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Biochemical Assay Optimization and Computational Screening Efforts to Identify Potential LuxS Inhibitors

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This thesis is written under the guidance of the Faculty Advisory Committee and, approved by all its members, has been accepted in partial fulfillment requirements for the degree of

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Abstract

Quorum sensing (QS) is a process of coordination of bacterial gene expression in response to cell population. System two QS is regulated by the small signaling molecule autoinducer-2 (AI-2) and is implicated in the infectious behaviors of various bacterial species. AI-2 is biosynthesized from *S*-ribosylhomocysteine (SRH) by the enzyme LuxS and induces interspecies cell-to-cell communication. Inhibition of LuxS would therefore inhibit interspecies QS. Herein, a search for novel molecular species that will competitively bind with SRH in the LuxS binding site is performed *in silico*. Computational screening results are then validated *in vitro* using an optimized LuxS inhibition assay.

Chapter 1: Introduction

Quorum sensing (QS) is a process of coordination of bacterial gene expression in response to cell population. There are two different quorum sensing (QS) systems. System One quorum sensing is regulated by a class of signaling molecules called autoinducer-1 (AI-1) and is involved in intraspecies communication, occurring only within one species. System Two quorum sensing is regulated by a small signaling molecule called autoinducer-2 (AI-2). AI-2 is synthesized from *S*-D-ribosyl-L-homocysteine (SRH) by the enzyme LuxS and induces interspecies cell-to-cell communication in many human pathogens.[1–3] AI-2 is implicated in the infectious behaviors of various bacteria that cause a range of human diseases, including those shown in Figure 1.1.[4] Inhibition of LuxS would thereby potentially inhibit interspecies quorum sensing.

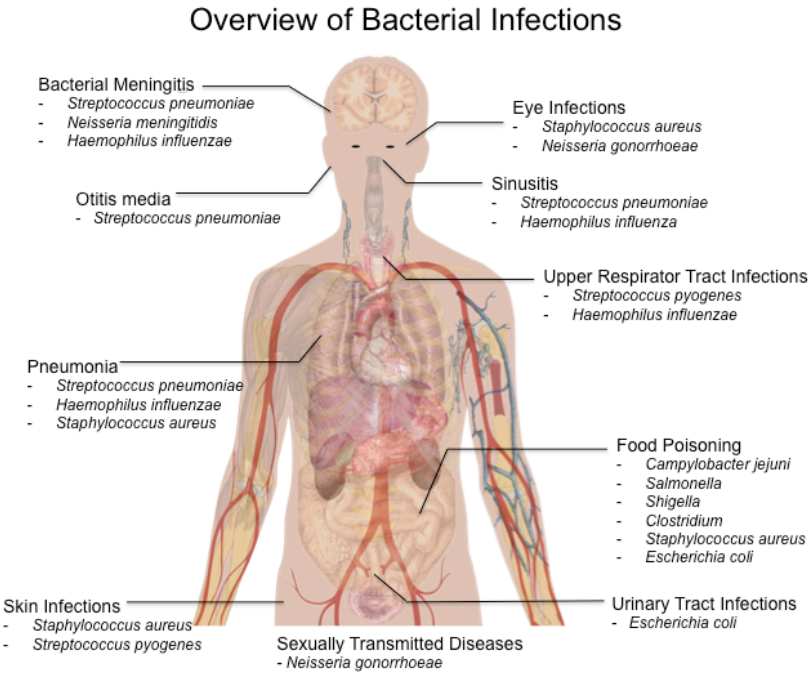

Figure 1.1 | Quorum Sensing and Disease. List of bacterial infections involved in quorum sensing that directly affects humans in different areas of the anatomy.[4]

Currently, a number of inhibitors of LuxS have already been discovered. Many of these inhibitors, such as *(*S*)*-2-amino-4-(((2*S*,3*R*)-2,3-dihydroxy-4-(hydroxyamino)-4-oxobutyl)thio)butanoic acid reported by Wnuk *et al*. that imitate the physical traits of SRH, were developed by a rational design approach (Figure 1.2).[1] Another type of LuxS inhibitor that has also been reported was discovered in nature, which is the brominated furanone (Figure 1.2).[2] The unique structure of the brominated furanone as a competitive inhibitor of LuxS gave the idea for the discovery of more "non-SRH analogs" for LuxS inhibition. Herein, a search for novel molecular species that will competitively bind with SRH in the LuxS binding site is performed *in silico*. The program used for the search for these novel LuxS inhibitors is DOCK Blaster. The *in silico* results are then tested to determine whether LuxS activity is inhibited *in vitro* using an optimized LuxS inhibition assay.[3]

SRH Analog **Non-SRH Analog**

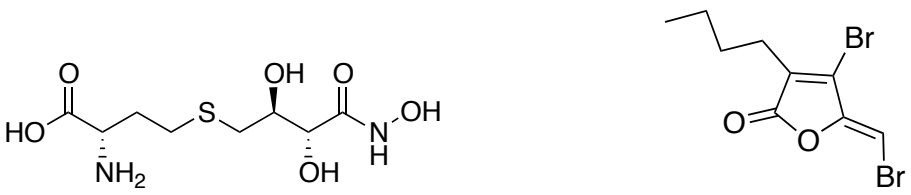

Figure 1.2 | LuxS Inhibitors. Examples of the two different types of known LuxS inhibitors. The SRH analog *(S)*-2-amino-4-(((2*S*,3*R*)-2,3-dihydroxy-4-(hydroxyamino)-4-oxobutyl)thio)butanoic acid reported by Wnuk *et al*. is an example of rationally-designed LuxS inhibitor.[1] The non-SRH analog, a brominated furanone, is the inspiration for the search for more non-SRH analogs of LuxS *in silico*.[2]

1.1 | *S*-Ribosylhomocysteinase (LuxS). The LuxS enzyme from the bacterium *Bacillus subtilis* is a homodimer. Its activity is greatly dependent on the appropriate binding of its substrate, *S*-D-ribosyl-L-homocysteine (SRH) to key amino acids. LuxS contains a Fe^{2+} metal ion as a cofactor (and not a Zn^{2+} cofactor that was previously reported), which is connected to

a water molecule tetrahedrally with the residues His-54, His-58, and Cys-126.[5,6] Although LuxS was determined to be a Fe^{2+}–containing protein in nature, the enzyme also functions with Co^{2+} as its metal cofactor *in vitro*.[5–9]

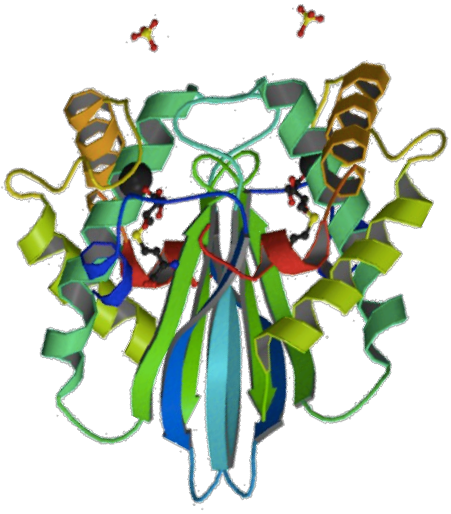

Figure 1.1.1 | LuxS. The X-ray crystal structure of the LuxS homodimer enzyme from *B. subtilis* with a cobalt cofactor (Co-BsLuxS) containing *S*-ribosylhomocysteine (SRH).[6] Its molecular weight is approximately 36 kDa.

1.2 | *S*-D-Ribosyl-L-homocysteine (SRH). *S*-D-Ribosyl-L-homocysteine (SRH) is the substrate for the LuxS enzyme. In solution, SRH can take on one of two forms: a cyclic (closed-form) or linear (open-form; Figure 1.2.1). Both of these variants were studied and used for the docking of the enzyme Co-BsLuxS-HT.

Closed-SRH **Open-SRH**

Figure 1.2.1 | SRH Structural Variants. *S*-D-Ribosyl-L-homocysteine (SRH) can exist in either of two structural forms.

LuxS crystal structures bound to both the cyclic and linear forms of SRH may be found in the Protein Data Bank (PDB): structure 1JVI has cyclic SRH bound, and structure 1YCL has the 2-ketone intermediate (of the SRH to AI-2 reaction) bound.[5,6] Both forms were used for docking because, according to Huang *et al.*, SRH may bind to the LuxS enzyme when its ribosyl moiety is in either its α- and β-SRH form or as a linear aldose. The binding of α-SRH is more thermodynamically preferred because of its low binding energy requirement of 47.5 kJ/mol, which is then followed by the β-SRH. The ring-opening process resulting in the 2-keto-SRH intermediate was due in part by the assisted ring opening by H_2O at the active site.[5,6,8]

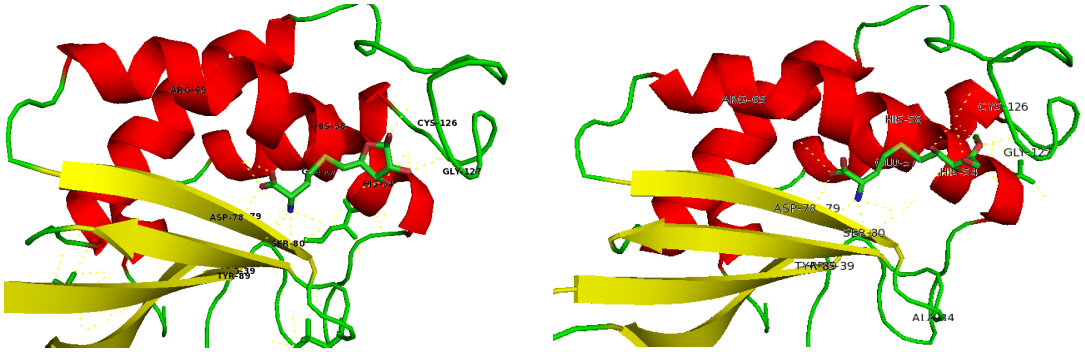

Figure 1.2.2 | SRH variants bound to LuxS. Image of the 1JVI (left) and 1YCL (right) BsLuxS crystal structures showing the open and closed representation forms of SRH bound in each active site. Picture was obtained using PyMOL.

1.3 | Biochemical Analysis of LuxS Activity. The activity of the Co-BsLuxS-HT can be monitored using a biochemical assay called the Ellman's assay. It is a colorimetric assay that monitors free thiols in solution by producing a yellow color in the presence of such thiols.[1,3,10]

1.3.1 | Co-BsLuxS-HT. The enzyme LuxS is a homodimeric enzyme responsible for the production of autoinducer-2. The enzyme Co-BsLuxS-HT was isolated from the bacterium *Bacillus subtilis (B. subtilis)*, whose protein-specific sequence was inserted into the *Escherichia coli (E. coli)* pET22b(+) vector. The pET22b(+) vector has a C-terminal six-histidine tag sequence and an ampicillin resistance marker. This plasmid came to be eventually known as ELS1409, which was provided by the Bassler lab (Princeton University). The isolated vector was freshly transformed into BL21[DE3] Gold competent cells for the expression of the Co-BsLuxS-HT protein. Since the vector has an ampicillin marker, all bacterial cultures were grown with 75 mg/mL ampicillin so that the only bacteria able to grow were those with ampicillin resistance. The Co-BsLuxS-HT protein isolated from the bacterial growth was then purified using a cobalt column because the six-histidine tag has an affinity for cobalt.[5,10]

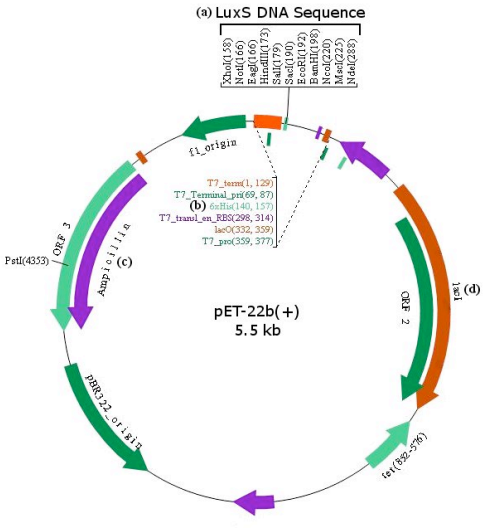

Figure 1.3.1 | pET22b(+) vector. The plasmid pET22b(+) was used to insert the DNA sequence that is to be used for the expression of the Co-BsLuxS-HT protein. **(a)** Vector region of DNA encoding *B. subtilis* LuxS **(b)** C-terminus six-histidine residues **(c)** Ampicillin marker and **(d)** location of the *lacI* repressor gene.[11]

1.3.2 | Ellman's Assay. The Ellman's assay is generally used for the quantification of free thiols in a solution. The thiols react with Ellman's reagent 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB), which undergoes a disulfide bond exchange with a thiol molecule and forms the product 2-nitro-5-thiobenzoate (NTB^-), which has a yellow color. During the performance of the assay, the yellow product is monitored at 412 nm using the UV–Vis spectrophotometer.[1,3,7,9] For LuxS, the Ellman's assay is used to indirectly determine how much autoinducer-2 is produced. SRH binds to the LuxS enzyme and produces DPD (the AI-2 precursor) and homocysteine at a 1:1 ratio. The homocysteine is then converted into a disulfide conjugate of homocysteine and DTNB, and the yellow product 2-nitro-5-thiobenzoate is monitored at 412 nm. Since the 2-nitro-5-thiobenzoate is produced at a 1:1 ratio with DPD, for every one 2-nitro-5-thiobenzoate produced, one autoinducer-2 is also produced.[1,10,12]

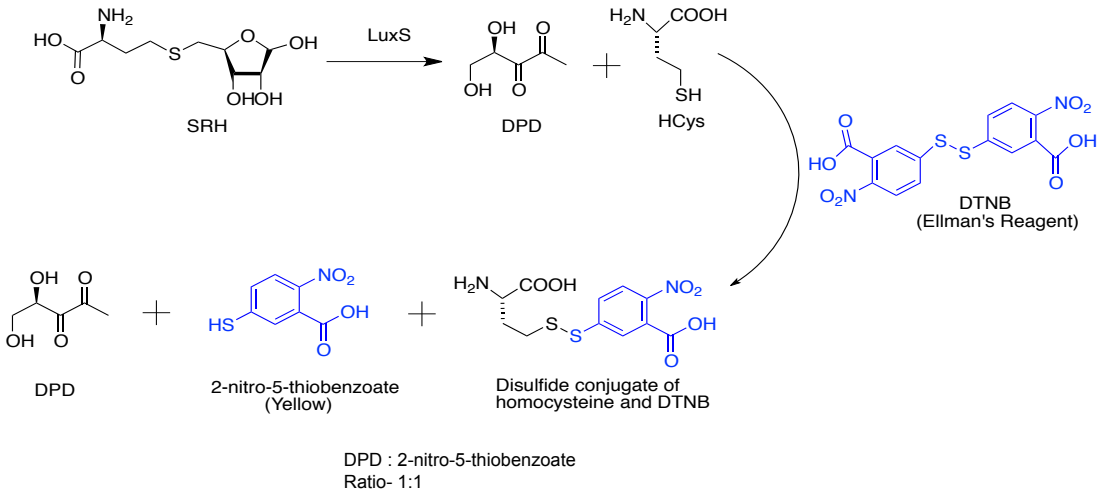

Figure 1.3.2 | Ellman's Assay for LuxS Activity. The Ellman's assay reaction from the conversion of SRH to DPD and homocysteine by the enzyme LuxS. Homocysteine reacts with DTNB to produce the yellow colored product, 2-nitro-5-thiobenzoate. Its production is at a 1:1 ratio with DPD; hence one autoinducer-2 is also produced.[5,12,13]

1.3.3 | Inhibition Assay. The type of inhibition that is the focus of this project is reversible inhibition. Reversible inhibition of an enzyme may occur in many different ways. The three types of reversible inhibition are uncompetitive, non-competitive, and competitive inhibition. Uncompetitive inhibition is when the inhibitor binds to a different site other than the enzyme active site so that it does not compete or interfere with the binding of the substrate and only occurs when the enzyme–substrate complex has been formed. Non-competitive inhibition binds also at a site different from the enzyme active site. However, when the inhibitor is bound, it alters the enzyme conformation and reshapes the active site, which does not allow for full functionality. Competitive inhibition is defined as a substrate having equal or greater affinity than the substrate for the enzyme active site. The inhibitor competitively competes with the substrate for the enzyme active site. The reason why competitive inhibitors were chosen over other types of inhibitors for this project is because most drugs that are currently produced are competitive inhibitors. Furthermore, competitive inhibitors are more straightforward to evaluate *in vitro*.[14] Increasing concentrations of the inhibitor are added to the Ellman's assay with a constant substrate (SRH) concentration.[1–3] As the concentration of inhibitor increases, it is less likely for the substrate to compete for the active site, which ultimately decreases the enzyme activity.

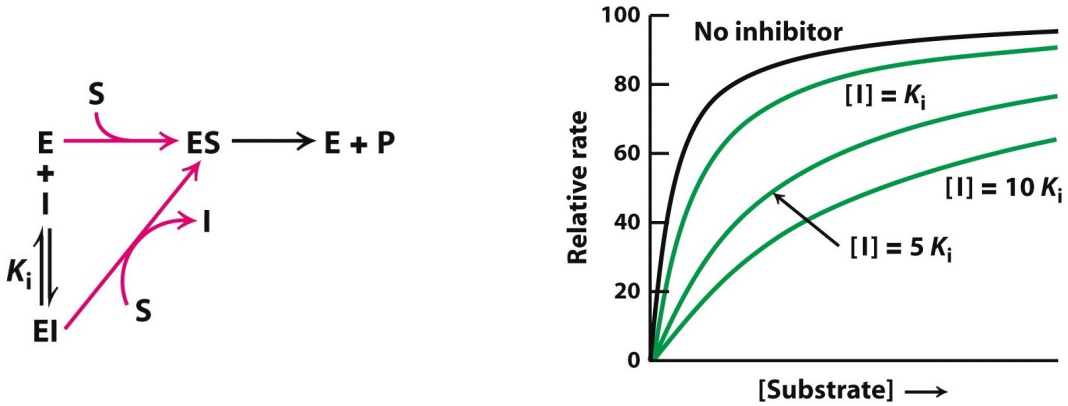

Figure 1.3.3 | Competitive Inhibition. Above is an illustration of what is hoped to be accomplished by discovering potential new competitive inhibitors. Without any presence of an inhibitor the substrate can bind to enzyme active and react to form products without any disruption. However, when as an increase amount of competitive inhibitor is added, the rate of reaction should start to decrease, but this is dependent upon how potent the inhibitor is.[1-3,14] (Figure modified from Reference 14.)

1.4 | Computational Screening. Computational screening is an increasingly widely used method for the discovery of new potential enzyme inhibitors. The program used is called DOCK Blaster.[15,16] The PDB code of an enzyme can be input into the DOCK Blaster program and docked with a selected database of small molecules that is provided by the program. The results are then viewed on PyMOL to check whether the potential inhibitors are indeed docked into the enzyme active site.[15,17] If so, an *in vitro* inhibition assay is then performed to validate the activity of each computational "hit" and to check the potency of the inhibitor.

1.5 | Summary of Project Goals. The goal of the project is to use the DOCK Blaster computational screening platform to discover new potential inhibitors that would competitively bind to the LuxS enzyme active sites, and then to validate the activity of these compounds using an optimized Ellman's assay. The discovery of competitive inhibitors of

LuxS is of great importance because of their potential to inhibit system-2 quorum sensing. Since system-2 quorum sensing is involved in interspecies communication of bacteria in many human pathogens, inhibiting the reaction that produces autoinducer-2 that is involved in signaling communication amongst the bacteria is an ultimate goal for potential therapeutic drugs. First, an optimization of the Ellman's assay for the eventual biochemical evaluation of potential inhibitors was achieved (Chapter 4). Second, various parameters for the DOCK Blaster computational screening program, including the choice of a suitable LuxS crystal structure (1JVI), were investigated. A number of computational screening runs were executed, with the predicted binding interactions of "hit" compounds visualized using PyMol (Chapter 5). A subset of compounds predicted to bind with high affinity to the LuxS active site was then evaluated using the Ellman's assay to determine whether or not these compounds truly inhibited LuxS *in vitro* (Chapter 6).

Chapter 2: Materials and Methods

2.1 | Purification of Co-BsLuxS-HT. The purification of the Co-BsLuxS-HT enzyme is modified from literature reports as discussed below.[7,10]

2.1.1 | Chemical Reagents. Cobalt chloride ($CoCl_2$), sodium chloride (NaCl), ethanol, manganese chloride ($MnCl_2$), borate (H_3BO_3), ammonium molybdate ($(NH_4)_6Mo_7O_{24}$), copper sulfate ($CuSO_4$), hydrogen chloride (HCl), D-glucose, ammonium sulfate [$(NH_4)_2SO_4$], potassium phosphate dibasic (KH_2PO_4), and potassium phosphate monobasic (K_2HPO_4) were purchased from commercial vendors.

2.1.2 | Biochemical Reagents, Kits, and Supplies. Casamino acids and LB agar were purchased from BioWorld. BL21[DE3] Gold competent cells were purchased from Agilent Technologies. Ampicillin sodium salt and the vacuum filtration systems were purchased from VWR. 5 mL cobalt columns were purchased from GE Healthcare. TALON® Resin was purchased from Clontech laboratories. PageBlue™ protein staining solution, Tris EDTA (TE) buffer, PageRuler™ plus prestained protein ladder, bovine serum albumin (BSA) standard kit, and Bradford dye were purchased from Fermentas.

2.1.3 | Purification of Co-BsLuxS-HT. The purification of Co-BsLuxS-HT enzyme was originally described in the literature by Pei *et al.*[7] Modifications to this literature procedure

were implemented to optimize LuxS production, including the use of French press and the ÄKTAprime Plus protein purification system.[10]

2.1.4 | Preparation of Reagents. The reagents required for the purification of the Co-BsLuxS-HT enzyme are prepared as directed: **(A)** Co-BsLuxS-HT Minimal Media (1 L). First, 1000X trace metals solution was prepared by adding 500 mM $MgSO_4$, 0.5 mM NaCl, 0.1 mM $MnCl_2$, 0.5 mM H_3BO_3, 1 µM $(NH_4)_6Mo_7BO_3$, and 10 µM $CuSO_4$ into 10 mL of 0.1 M HCl. The solution was then filtered through a Stericup. The 100X sugar stock solution was prepared by adding 25% D-glucose and 10% $(NH_4)_2SO_4$ in hot distilled water. Then, once the solution was warm to the touch, 200 µg/mL thiamin and 100 µg/mL D-biotin were added into a final volume of 500 mL distilled water and filtered through a Stericup. For the preparation of 1 L LuxS minimal media, 5.5 g KH_2PO_4, 10.8 g K_2HPO_4, 10 g NaCl, and 5 g casamino acids were mixed into 989 mL distilled water. Then, 1 mL of 1000X trace metals stock solution and 10 mL of 100X sugar stock solution were added to the solution. The media was then filtered through a 0.45 µM pore size Stericup. **(B)** LB Amp^{75} Media (1 L). To 800 mL H_2O was added 10 g bacto-tryptone, 5 g yeast extract, 10 g NaCl. Then, the pH was adjusted to 7.5 with NaOH with a final volume of 1 L and autoclaved for 15 min. at 121 °C. After media has cooled, 750 µL of 100 mg/mL ampicillin was added to the media for a final antibiotic concentration of 75 mg/mL. **(C)** 100 mM $CoCl_2$ (50 mL). 0.65 g of $CoCl_2$ was added to 50 mL distilled water and stored at room temperature for future use. **(D)** 100 mM Isopropyl β-D-1-thiogalactopyranoside/IPTG (50 mL). 1.19 g of IPTG was added to 50 mL of distilled water and mixed on a magnetic stirrer. 1 mL aliquots were prepared and kept at –80 °C for future use. **(E)** Preparation of the Bacterial Glycerol Stocks. To prepare bacterial glycerol stocks, 750 µL of the bacterial cultures grown in LB Amp^{75} media were added to

250 µL of 80% glycerol in a 1.5 mL Eppendorf tube and quick-frozen with liquid nitrogen and stored for future use. **(F)** Buffers**.** Preparation of binding buffer included the addition of 50 mM sodium phosphate, 300 mM NaCl, and adjusting the pH to 7.4 with a final buffer volume of 1 L. For preparation of mechanical lysis buffer, a solution of 20 mM Tris·HCl (pH 8.0), 500 mM NaCl, and 5 mM imidazole (pH 8.0) was prepared. The Elution Buffer (EB) was made consisting of 20 mM Tris·HCl (pH 8.0), 500 mM NaCl, 150 mM imidazole (pH 8.0). For the preparation of 10X running buffer, 30.3 g of trizma base, 144.0 g of glycine, and 10 g of SDS were dissolved and mixed into 1 L of distilled water. **(G)** Preparation of 12.5% SDS-PAGE Gels. To make a 12.5% gel, a stacking gel solution and a separating gel solution were made. For preparation of the separating gel solution, 3.35 mL of distilled water, 2.5 mL of 1.5 M Tris·HCl (pH 8.8), 55 µL of 20% SDS, 4 mL of Acrylamide/Bis, 80 µL of 10% ammonium persulfate (APS) were added. 8 µL of tetramethylethylenediamine (TEMED) was added last. For the stacking gel solution, 6.1 mL of distilled water, 2.5 mL of 0.5 M Tris·HCl (pH 6.8), 55 µL of 20% SDS, 1.3 mL of 30% Acrylamide/Bis, 80 µL of 10% APS were added. 8 µL of TEMED was added last. Bio-Rad Mini-PROTEAN® Tetra handcast gel frame for two gels was set up. First the separating gel solution was poured up to 3/4th height of the gel frame and allowed to solidify. Isopropanol was immediately added to level the surface of the gel. After the gel has solidified (as could be seen from the leftover solution) the isopropanol was removed. The stacking gel was then added into the remaining space and combs were placed at the top of the gel frame. Once the gel has solidified, it was stored in the refrigerator by wrapping it in wet paper towels and keeping it in a Ziploc bag.

2.1.5 | Protein Purification. A 5 mL LB Amp[75] media starter culture containing the *E. coli* BL21 bacterial cells freshly transformed with plasmid ELS1409 was grown overnight for

approximately 16 h at 37 °C. The 5 mL starter culture was then back-diluted 1:1000 into 50 mL minimal Amp^{75} media and grown for 16 h at 37°C. The 50 mL culture in turn was back-diluted 1:50 into 2 L of minimal Amp^{75} media and grown until its OD_{600} has reached ~0.6 at 37 °C. Final concentration of 200 μM $CoCl_2$ was then added to the culture and incubated for 30 min. at 30 °C. After 30 min., the cultures were induced with final concentration 100 μM IPTG. The cultures were incubated with shaking for another 16 h at 30 °C. After 16 h, the pellet of cells was collected by centrifugation of the media at 5000 rpm and a temperature of 4 °C. The pellet was resuspended in 35 mL mechanical lysis buffer. The resuspended cells were lysed via French press at approximately 20,000 psi and then centrifuged at 5000 rpm in order to retrieve the supernatant that contains the Co-BsLuxS-HT protein. The supernantant containing the Co-BsLuxS-HT protein was sequentially filtered using 5.0 μM, 0.8 μM, and 0.2 μM filter units in order to make certain that the column will not clog because of the denseness of the supernatant. The Co-BsLuxS-HT protein was purified using a 5 mL TALON® Crude HiTrap, which was connected to run using the ÄKTAprime plus protein purification system. The 5 mL Talon® Crude HiTrap was initially equilibrated with 5 column volumes (CV) of Binding Buffer. The ÄKTA contains already set programs for affinity columns, which was used for the purification of the Co-BsLuxS-HT protein. The "Affinity Gradient Step" was chosen from "Methods Template" on the ÄKTA. The template was programmed line-by-line in accordance to the template. First, set sample injected by "pump". Pressure limit is set to 0.5 MPa, flow rate is set to 4 mL/min, and the fraction size is set to 1 mL. The equilibration volume is set to 3 CV because the column was initially already equilibrated with 5 CV during the cleaning process. The sample inject volume used is dependent upon the amount of protein supernatant left from the filtration with the addition of 5 mL of extra volume using the binding volume after the only very little protein supernatant

has already been pumped into the system. Then, the "wash 1" volume is set to 5 CV and the elution volume is set to another 5 CV. No "wash 2" volume is needed and therefore, when asked if method is ready, "yes" was selected. The Co-BsLuxS-HT protein (colored purple) was eluted out automatically and fractions 4–12 was collected based on the peak.[10] The concentration of the protein was determined by measuring the absorbance of each fraction using the NanoDrop Spectrophotometer ND-1000. The Abs. 1% is set to 6.23, which was determined from the LuxS sequence using the ExPASy ProtParam tool. Then, some fractions were eliminated based on the amount of Co-BsLuxS-HT needed to perform the LuxS inhibition assay (160 μM working solution required). Fractions containing sufficient LuxS were pooled and a triplicate NanoDrop concentration reading taken. Then, 80 μL of combined fractions were then aliquoted into 1.5 mL Eppendorf tubes and stored for future use in the performance of activity assays and inhibition assays.

2.1.6 | Determination of Protein Concentration by Bradford Assay. Initially the concentration of Co-BsLuxS-HT was determined by Bradford assay.[18] Bradford assay is a colorimetric protein assay that measures the Coomassie Brilliant Blue G-250 dye that binds to the protein at 595 nm. In order to measure the concentration of the Co-BsLuxS-HT proteins, a standard curve run was done using different concentrations of bovine serum albumin (BSA). The BSA standard values can be seen below (Table 2.1.6). Transparent 96-well plates from Costar (part no. 3598) were used to perform the Bradford assay. For consistency, the standards were run in triplicate. Each well contained 300 μL of the Coomassie Brilliant Blue G-250 dye along with the different concentrations of BSA standards. The 96-well plate was ran using the EnVision® multlilabel reader, which was programmed to measure the absorbance at 595 nm with an 8 min delay. The delay was programmed to 8 min in order to give the dye enough

time to bind with the proteins to give the dye enough time to bind with the proteins to produce the blue color.[18]

Table 2.1.6 | Bradford Assay Standards.[a]

BSA standard concentration (mg/mL)	Volume of BSA standard (µL)	Amount of BSA (µg)
0	0	0
0.125	4	0.5
0.25	6	1
0.25	4	1.5
0.5	5	2
0.5	4	2.5
0.75	4.7	3
0.75	4	3.5
1	4	4

[a] The table shows the amount of BSA used in order to create the standard curve. From this, a linear equation was obtained and used for the calculation of the Co-BsLuxS-HT protein concentration by solving for "x" using absorbance as "y".[18,19]

2.1.7 | Determination of Protein Concentration using NanoDrop Spectrophotometer ND-1000. The NanoDrop spectrophotometer is now the primary method to determine the concentration of the Co-BsLuxS-HT protein. The LuxS sequence of the protein was inputted into the ProtParam program by ExPASy online. The result given includes the molecular weight of the enzyme along its extinction coefficient (11460 M^{-1} cm^{-1}), and the Co-BsLuxS-HT absorbance at 1%, which is 6.23. The absorbance at 0.1% was input into the NanoDrop spectrophotometer program, which was set to read at A_{280}. A blank was run using the elution buffer; only 2 µL of the buffer is needed. After the blank, 2 µL of the Co-BsLuxS-HT was

measured using the NanoDrop spectrophotometer. The instrument was blanked three times and the Co-BsLuxS-HT was measured three times for consistency purposes.

2.1.8 | SDS-PAGE. After purification of the Co-BsLuxS-HT protein, an SDS-PAGE gel was run to assess the purity of the protein. The pre-made 12.5% SDS-PAGE gel was set in the cell buffer dam, facing inwards. Then, 1% running buffer was poured into the inner chamber until it overflowed the required level in the outer chamber. The Co-BsLuxS-HT was mixed with loading dye containing the reducing reagent dithiothreitol (DTT) and was heated in boiling water for 10–15 min. The gels were loaded with 2–4 μL of the dyed proteins and run at 150 V until the dye could be visually seen at the bottom of the glass plates. The gels were removed from the glass plates and placed in a container that is rocked/shaken with dH_2O to remove lingering running buffers (do 3X for 10 min each). Approximately 20 mL of PageBlue Protein Staining Solution by Thermo Scientific was poured onto the SDS-PAGE gel and the container shaken overnight. The next day, the gels were washed until the excess dye has been removed and the bands can be visually seen.

2.2 | Computational Screening using DOCK Blaster. Using DOCK Blaster, potential inhibitors of LuxS can be determined. DOCK Blaster is freely available online and can be completely automated, which makes it applicable to anyone interested in computational screening.[15–17] To use DOCK Blaster, a PDB code must be obtained from the Protein Data Bank website (http://www.rcsb.org/pdb/home/home.do). Under the search bar, enter the molecule name "*S*-Ribosylhomocysteine" and click on "*Bacillus subtilis"*. A list of all known LuxS structures from *B. subtilis* with SRH will be shown including their PDB codes. The PDB codes chosen are 1JVI (closed ring SRH) or 1YCL (opened ring SRH).[6,20] Next, the

DOCK Blaster site is opened (http://blaster.docking.org/). Under the "Dock" tab, click on "Start with a PDB code". Enter PDB code 1JVI (closed-form SRH) and specify its 3-letter ligand code under "Ligand", which is RHC (stands for Ribosylhomocysteine), then click on "Dock!". DOCK Blaster will submit a job and give a 5-digit query number. This job number will be the identification code for the particular structure that was docked. (This step can take up an hour or more before proceeding to the next step). Once "Calibration Docking" is complete, a score will be given. Calibration docking represents the results of re-docked ligand in a table. Green indicates good, yellow marginal, and red poor re-docking results. There are two different sampling schemes ("Coarser" or "Finer"), which are used to sample ligand orientations. There are also two different scoring schemes ("Amber" or "Polarized"), which use the partial atomic charges on a protein ("amber 94") or increase the dipoles on selected polar atoms in residues within 3.5 Å ("polarized") of the crystallographic ligand without changing the net charge.[15] Pockets of different sites will also be given and can be chosen as the site in which to dock potential inhibitors; however, the site which SRH binds to the LuxS is automatically chosen without the need to manually pick a pocket.[15,21] Since the goal of the project is to discover potential new competitive inhibitors for LuxS, docking can proceed after calibration. Docking begins by choosing the database type. Choose "By Vendor" and then under "Database (#) number of molecules" the vendor "kegg" is chosen. The KEGG database was chosen because it contains the closed-SRH form, which gives a positive control to the docking experiment. Sampling and scoring schemes can be chosen by either choosing sampling to run "Faster" or "Slower". There are also two types of scoring to choose from, the first one is "Scoring #1" or "Scoring #2". Once the selections for the schemes have been chosen, click on "Dock". Depending on whether the sampling scheme is fast or slow, the run can take a day or a few weeks for the results to be given. Once the results are given, it can be

viewed under "subset kegg". Click on "subset kegg"; this page will show the results for every possible inhibitor from the KEGG database that supposedly binds to the LuxS active site.[22] The substrates are ranked based on how well they scored. The more negative their score, the better the molecule is predicted to bind to the LuxS enzyme active site.[15,17] The substrate *S*-D-ribosyl-L-homocysteine (SRH) should have also re-docked as well (positive control). After docking is complete, a new dock can then be initiated. (You may only dock one database at a time). By clicking on the PyMOL tab on each of the possible inhibitors, each inhibitor can be viewed using PyMOL, which is a program that produces high quality 3D images of small molecules to large macromolecules such as proteins.[23] From PyMOL, it can be seen as to whether the potential inhibitor is indeed bound the LuxS active site *in silico*.[15]

2.3 | Ellman's Activity Assay. The Ellman's Activity assay is a colorimetric kinetics assay that measures the absorbance of a solution at 412 nm to indicate the presence of thiols. The general procedural requirements for using Ellman's assay to determine LuxS activity (in the absence of inhibitors) is derived from literature reports as described below.[5,10]

2.3.1 | Chemical Reagents. Ellman's reagent (5,5'-dithiobis-(2-nitrobenzoic acid) or DTNB), 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) sodium salt, and NaCl were purchased from commercial sources. SRH was prepared synthetically in our laboratory.

2.3.2 | Preparation of Ellman's Activity Assay Reagents. The methodology for the preparation of Ellman's assay reagents is as follows: (a) 5× Co-BsLuxS-HT Buffer (250 mL). 32.5 g of HEPES sodium salt (500 mM) was added into 250 mL of distilled water. The pH

was then adjusted to 7 with concentrated HCl. After pH has reached 7, 10.958 g of NaCl (750 mM) was added to the buffer solution. (b) Co-BSLuxS-HT (160 µM final working solution concentration – 150 µL working solution). The determined concentration of Co-BsLuxS-HT from the NanoDrop spectrophotometer was 321.1 µM. From the 321.1 µM stock of Co-BsLuxS-HT, a 160 µM working solution was made with a final volume of 150 µL. Add 30 µL of 5× Co-BsLuxS-HT buffer, 74.7 µL of 321.1 µM Co-BsLuxS-HT, and 45.3 µL of distilled water together in a 1.5 mL Eppendorf tube to obtain a final working stock concentration of 160 µM of Co-BsLuxS-HT. (c) *S*-Ribosylhomocysteine (SRH) (680 µM final working solution concentration – 1000 µL). 200 µL of 5× Co-BsLuxS-HT buffer, 34 µL of SRH (20 mM SRH stock) and 766 µL of distilled water were mixed together into a 1.5 mL Eppendorf tube. (d) Ellman's Reagent (DTNB) was prepared with a final working concentration of 15 mM with a final volume of 2 mL. It was prepared by adding 0.01189 g of DTNB into 2 mL of 5× Co-BsLuxS-HT buffer.[5,10]

2.3.3 | Ellman's Activity Assay of Co-BsLuxS-HT. All working solutions are kept separately until each assay run. Table 2.3.3 below shows the amount of reagents needed for each run. Directly into the 1 mL glass cuvette, add 200 µL of 5× Co-BsLuxS-HT buffer first. Then, add the appropriate amount of distilled water as listed on Table 2.3.3 and following after that, the appropriate amount of SRH is added (each assay run will having increasing amounts of SRH (0–68 µM final concentration in a cuvette)) in the cuvette. The 5 µL of Co-BsLuxS-HT is added to one side of the cuvette, making sure it is not immersed in solution yet, then add 10 µL of DTNB to the other side of the glass cuvette. Flip the glass cuvette over once with the cap that is provided with the cuvette and insert the cuvette into the Hewlett Packard B452A Diode Array Spectrophotometer quickly. The spectrophotometer is programed to read at 412

nm and record its readings every 17 seconds for a total of 204 seconds.[5,12] Make sure to let the diode array warm up its lamps before use for about 15–30 min before performing any run. Also, a blank run using the first mixture on Table 2.3.3 is required before continuing to run the Ellman's Activity assay.

Table 2.3.3 | Ellman's Activity Assay.[a]

[Final Concentration of SRH] (μM)	5× LuxS Buffer (μL)	dH_2O (μL)	680 μM SRH working solution (μL)	160 μM LuxS working solution (μL)	15 mM Ellman's Reagents (DTNB) (μL)
Blank	200	790	-	-	10
LuxS only	200	785	-	5	10
SRH only	200	690	100	-	10
1.060	200	783.4	1.56	5	10
2.125	200	781.9	3.13	5	10
4.250	200	778.8	6.25	5	10
8.500	200	772.5	12.5	5	10
17.000	200	760	25	5	10
34.000	200	735	50	5	10
68.000	200	685	100	5	10

[a] The first three runs in the Ellman's assay are controls. After the blank, LuxS only, and SRH only runs have been conducted, increasing concentrations of SRH are tested to determine the activity of the Co-BsLuxS-HT enzyme. The concentrations that are listed in the first column are the final concentrations of SRH in the 1 mL cuvette when the assay is performed. For each assay run, the initial rate of each run is recorded. The initial rates are then calculated to give the specific units μmol/mg/min.[7] They are then plotted using GraphPad Prism 5.0 in order to obtain the K_M, V_{max}, k_{cat}, and k_{cat}/K_M values.

2.3.4 | Kinetic Parameters Determination. The calculated specific activity was plotted onto GraphPad Prism 5.0. The specific activity was calculated using the extinction coefficient for DTNB (ε = 14,000 M^{-1} cm^{-1}), protein amount (mg), pathlength (1 cm), time (min.), and initial rates of the increasing concentration of SRH. A sample calculation can be seen below using a

sample initial rate of 2.20237×10^{-4}. Note that when the actual specific activity is plotted onto GraphPad Prism 5.0, the specific activity is plotted as 10^{-3} in order for easy comparability to the reports of Pei *et al*.[5,7]

$$2.2 \times 10^{-4} \frac{AU}{s} \left(\frac{mol \cdot cm}{14000\ L}\right)\left(\frac{1}{cm}\right)\left(\frac{60\ s}{min}\right)\left(\frac{10^6\ \mu mol}{L \cdot min}\right)\left(\frac{1\ L}{1000\ mL}\right)\left(\frac{1\ mL}{0.059\ mg}\right)$$

$$= 0.\ 016\ \mu mol/mg/min$$

A sample kinetics graph and its values can be seen on Figure 3.2.3.

2.4 | LuxS Inhibition Assay. The LuxS Inhibition assay is essentially the Ellman's Activity assay except with the addition of increasing amount of inhibitor holding the amount of SRH constant. Similar to the Ellman's Activity assay, the LuxS Inhibition assay is a colorimetric kinetics assay that measures the absorbance of a solution at 412 nm. Its general procedural requirements, as derived from literature reports,[7,10] are described below:

2.4.1 | Chemical Reagents. Ellman's reagent (5,5'-dithiobis-(2-nitrobenzoic acid or DTNB), 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) sodium salt, and NaCl were purchased from commercial vendors. SRH was prepared synthetically in our laboratory. [10]

2.4.2 | Preparation of LuxS Inhibition Assay Reagents. The LuxS Inhibition assay reagents are prepared as follows: (a) 5× Co-BsLuxS-HT Buffer (250 mL). 32.5 g of HEPES sodium salt (500 mM) was added into 250 mL of distilled water. The pH was then adjusted to 7 with concentrated HCl. After the pH had reached 7, 10.958 g of NaCl (750 mM) were added to the

buffer solution. (b) Co-BSLuxS-HT (160 μM final working solution concentration – 150 μL working solution). The determined concentration of Co-BsLuxS-HT from the NanoDrop spectrophotometer was 321.1 μM. From the 321.1 μM stock of Co-BsLuxS-HT, a 160 μM working solution was made with a final volume of 150 μL by adding 30 μL of 5× Co-BsLuxS-HT buffer, 74.7 μL of 321.1 μM Co-BsLuxS-HT, and 45.3 μL of distilled water together in a 1.5 mL Eppendorf tube. (c) *S*-Ribosylhomocysteine (SRH) (680 μM final working solution concentration – 1000 μL). Mix together 200 μL of 5× Co-BsLuxS-HT buffer, 34 μL of SRH (20 mM SRH stock), and 766 μL of distilled water into a 1.5 mL Eppendorf tube. (d) Ellman's Reagent (DTNB) was prepared with a final working concentration of 15 mM with a final volume of 2 mL by adding 0.01189 g of DTNB into 2 mL of 5× Co-BsLuxS-HT buffer.[5,7] (f) Inhibitors (Final working solution concentration 680 μM – 2000 μL). Inhibitors are initially prepared with a final concentration of 0.5 M. From the 0.5 M inhibitor stock, the 680 μM working solution was prepared by adding together 400 μL of 5× Co-BsLuxS-HT buffer, 1597.3 μL of distilled water, and 2.72 μL of 0.5 M inhibitor.[1–3,20,24]

Table 2.4.2 | Ellman's Inhibition Assay.[a]

Final Concentration of SRH] (μM)	5× LuxS Buffer (μL)	dH_2O (μL)	680 μM SRH working solution (μL)	160 μM LuxS working solution (μL)	680 μM Inhibitor (μL)	15 mM Ellman's Reagents (DTNB) (μL)
Blank	200	790	-	-	-	10
LuxS [34 μM] only	200	780	-	10	-	10
SRH [68 μM] only	200	690	100	-	-	10
LuxS [34 μM] + SRH [68 μM]	200	680	100	10	-	10
LSI [68 μM]	200	580	100	10	100	10
LSI [136 μM]	200	480	100	10	200	10
LSI [204 μM]	200	380	100	10	300	10
LSI [272 μM]	200	280	100	10	400	10
LSI [340 μM]	200	180	100	10	500	10

[a] The first four runs in the inhibition assay are controls. After the blank, LuxS only, SRH only, and LuxS + SRH runs have been conducted, increasing concentrations of inhibitor are tested to determine the inhibition of the Co-BsLuxS-HT enzyme. The inhibitor concentrations are listed in the first column with a final volume of 1 mL in the cuvette when the assay is performed. For each assay run, the initial rate of each run is recorded. The initial rates are calculated to give the specific units μmol/mg/min.[7] They are then plotted the same way as the Ellman's assay using GraphPad Prism 5.0 in order to obtain the K_M, V_{max}, k_{cat}, and k_{cat}/K_M values.

2.4.3 | IC_{50} Determination for Selected Inhibitors. The IC_{50} values were determined for substrates that showed initial promise in inhibition of the LuxS enzyme from the Ellman's Inhibition Assay (Section 2.4.2). It was imperative that the IC_{50} was determined so that it can

be seen how much of the potential inhibitor is needed to inhibit the LuxS enzyme by fifty percent. This was accomplished by using the initial rate of each assay to calculate the percent activity of the enzyme as increasing amounts of potential inhibitor is added to the Ellman's assay. The IC_{50} values of Argininosuccinate and L-NAME were calculated and determined. Equation for the calculation of the IC_{50} is shown below.

$$\%\ Inhibition\ = \left[\frac{(normal\ activity\ -\ inhibited\ activity)}{(normal\ activity)}\right] \times 100\%$$

$$100\ -\ \%\ inhibition\ =\ \%\ Activity$$

Table 2.4.3 | Determination of IC_{50} Values.[a]

Sample I.D.	Corrected LuxS Absorbance (A_{280})	% Inhibition	% Activity
LuxS [1.6 μM] + SRH [34 μM]	36	0	100
LSI [68 μM]	30	–0.17	83
LSI [136 μM]	22	–0.38	62
LSI [204 μM]	20	–0.45	55
LSI [272 μM]	11	–0.70	30
LSI [340 μM]	–2.1	–1.1	–5.8

[a] Process of how the % Activity was determined and eventually was graphed with the y-axis as % Activity and the x-axis is the log of inhibitor concentrations 68 μM, 136 μM, 204 μM, 272 μM, and 340 μM.

Chapter 3: Spectrophotometry

3.1 | Spectrophotometers. Spectrophotometry is widely used in biochemical analysis to calculate the concentration of a substance in solution. Spectrophotometry is defined as the measurement of color within a solution by observing the quantity of light being absorbed using a spectrum of ultraviolet, infrared, or visible light.[14,25,26] A wavelength (λ) is defined as the distance between adjacent peaks in a wave.[14.26] Each compound has a different characteristic wavelength of light that it absorbs. For example, when a solution appears green, it is because the blue and yellow light is being transmitted and the red light is being absorbed by the compound in solution. The relationship that connects the absorption of light to the concentration of the absorbing solution is determined using the Beer–Lambert Law. The Beer–Lambert Law equation is defined as:

$$A = \varepsilon \times \ell \times c$$

where A is absorbance and has no unit. Epsilon (ε) is the extinction coefficient and has the units M^{-1} cm^{-1}. The pathlength (l) is in units of cm and is usually assumed to be 1 cm unless otherwise suggested.[14,25,26] The concentration (c) has a unit that is the reciprocal of the epsilon unit.[25,26] This equation is typically used to determine the concentration of a compound in solution. In cases where the extinction coefficient ε is unknown, a standard curve methodology may be used.

In biochemistry, spectrophotometry is a useful technique for both determining the concentration of proteins and small molecules in solution and also for studying enzyme kinetic activity using the Michaelis–Menten equation. Here, spectrophotometry allows for

the determination of the enzyme activity by relating the reaction rate (V) of the enzyme obtained from the spectrophotometer to the substrate [S] concentration in the presence of an enzyme of interest.[26]

$$V_0 = V_{max} \frac{[S]}{[S] + K_M}$$

The Michaelis–Menten constants K_M, k_{cat}, and V_{max} can be automatically determined using graphing programs such as GraphPad Prism when the specific activity of the enzyme is graphed against the substrate concentration.

3.1.1 | PerkinElmer EnVision 2104 Multilabel Reader. The EnVision Multilabel Plate Reader instrument was initially used in this project to perform the Bradford assays in order to determine protein concentration.[18] Here, the EnVision is programmed to read absorbance at 595 nm using the 96-well plate. Using the 96-well plate allows for triplicate readings in just a single run and in less than 20 seconds.

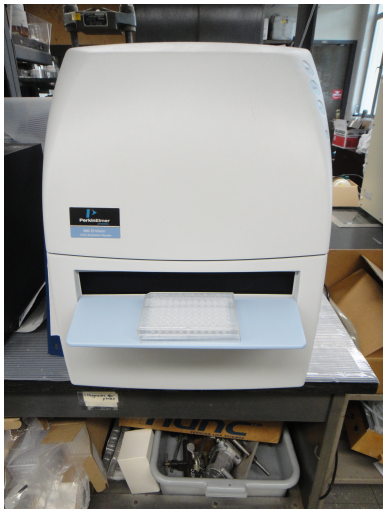

Figure 3.1.1 | EnVision Plate Reader. Image of the PerkinElmer Envision 2104 Multilabel Plate Reader and 96-well plate that is used to perform Bradford assays.

3.1.2 | NanoDrop Spectrophotometer. The NanoDrop spectrophotometer is another type of spectrophotometer instrument that measures the absorbance of light by a compound of interest. The NanoDrop spectrophotometer is usually used to determine the concentration of DNA. However, it can also be used to accurately determine protein concentration using the "other protein (E1%)" option.[27] For proteins of known primary sequence that are purified, concentration can be quantified by reading absorbance at 280 nm. The result is given in mg/mL and the concentration of the protein can be accurately determined up to 100 mg/mL without dilution. The NanoDrop spectrophotometer is best used to test samples of volumes as little as 1 µL, however, when dealing with proteins, 2 µL of volume is recommended in order for accurate reading of the protein concentration.[27]

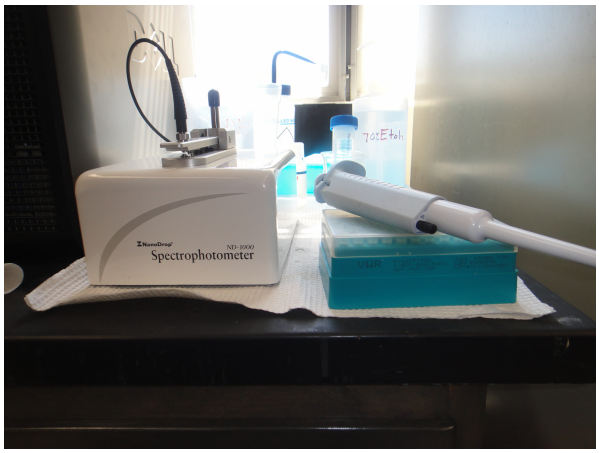

Figure 3.1.2 | NanoDrop Spectrophotometer. A picture of the NanoDrop Spectrophotometer ND-1000 and P-2 pipetman that is used for protein concentration determination.

3.1.3 | Agilent 8453 UV–Vis Spectrophotometer. The Agilent 8453 UV–Vis spectrophotometer uses diode array technology. The photodiode array contains 1024 individual photodiodes and the control circuits are within the semiconductor chip. The Agilent 8453 UV–Vis has a wavelength range between 190 nm and 1100 nm. The UV–Vis

spectrophotometer can be used to read at a fixed wavelength or multiple wavelengths. It can also be used to run biochemical analyses such as running a kinetics assay. The UV–Vis can run a kinetics assay by programming the program to run time-dependently and to be able to take measurements at specific time intervals in the assay.

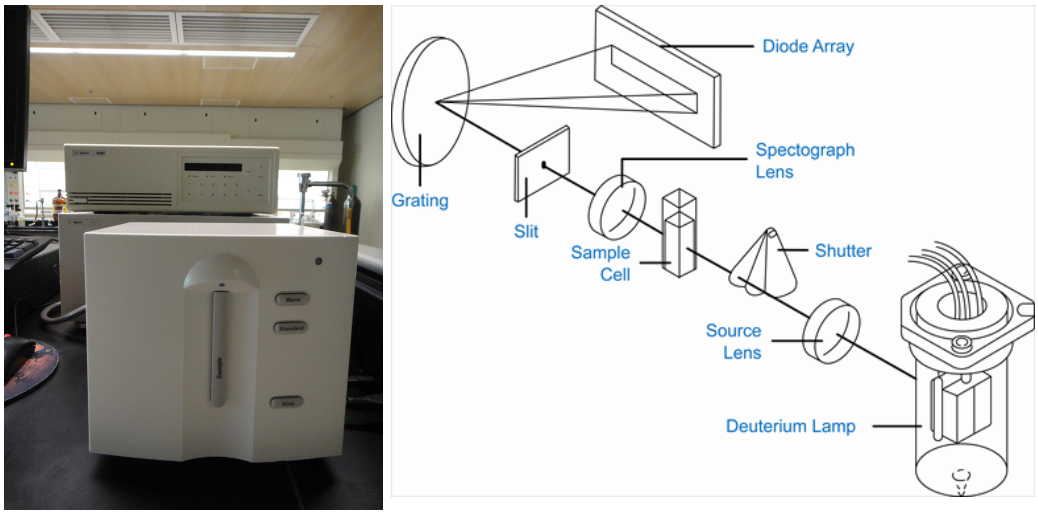

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Figure 3.1.3 | UV–Vis Spectrophotometer. (Left) Image of Agilent 8453 UV–Vis spectrophotometer. (Right) Diagram of typical UV–Vis spectrophotometer anatomy that shows how the light source hits the cuvette containing the sample.[14,25,26]

3.2 | Spectrophotometric Assays. UV–Vis spectrophotometry may be used to study enzymes by determining protein concentrations and the concentrations of other analytes in solution such as the products of an enzymatic reaction. Depending on the type of cuvette that is being used, a total volume of 1 mL is usually needed in order for instruments such as the Agilent UV–Vis spectrophotometer to be able to read absorbances. However, for instruments such as the EnVision Multilabel Plate Reader by PerkinElmer, only 600 µL of volume is needed for each well in a 96-plate well. For the NanoDrop spectrophotometer instrument, 2 µL of volume is all that is needed to read the concentration of the solution.

3.2.1 | Bradford Assay. The Bradford assay is a type of biochemical analysis used to determine the concentration of a protein in solution, which was developed by Marion M. Bradford. It is a colorimetric assay in which the absorbance is being measured at 595 nm. The protein solution turns more intensely blue as proteins bind to the Coomassie Brilliant Blue G-250 dye. In acidic conditions, the proteins will bind to the Coomassie Brilliant Blue G-250 dye, which then turns the dye blue. The original red color is due in part to the dye being acidic and doubly protonated in a cation form. When the protein is bound the solution turns blue, which is stable and unprotonated. The blue color is the result of basic amino acids such as histidine, lysine, and mainly arginine binding to the dye.[18,19] The binding of proteins to the dye is caused by van der Waals forces and hydrophobic interactions. Hydrophobic interaction is defined as the interaction between non-polar hydrocarbons that are forced to bind to each other by the surrounding water interactions.[14] These are prevalent within the Bradford assay because the protein that binds to the Coomassie Brilliant Blue G-250 dye has a native tertiary structure that is disrupted by the donation of a free electron by the Coomassie Brilliant Blue G-250 to an ionizable group within the protein, which reveals the hydrophobic pockets within the enzyme.[18,19] Van der Waals interaction is defined as a weak force attraction between non-polar molecules that is caused by a change in dipole moment. Van der Waals interactions are much weaker than chemical bonds.[14] In regards to the Bradford assay, van der Waals forces influences the binding of the hydrophobic pockets of the enzyme to the Coomassie Brilliant Blue G-250 dyes' non-polar region by non-covalent binding. In order to determine the concentration of a protein by the Bradford method, a standard curve is first created using increasing known concentrations of the commodity protein BSA. The absorbance of a solution of the protein of interest is evaluated against the equation of the

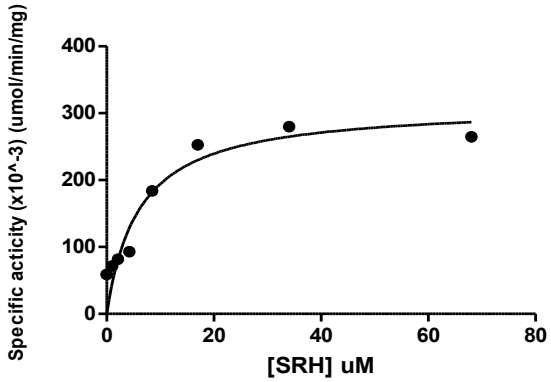
standard curve line. The sample solution may require dilution in order to fit into the standard curve.

3.2.2. NanoDrop Spectrophotometer. The concentration of a purified enzyme can presently be determined more directly using the NanoDrop spectrophotometer ND-1000. From the NanoDrop spectrophotometer menu, "Protein A_{280}" was chosen. Once the screen opens, "other protein (E1%)" is where the absorbance at 1% is input. To obtain absorbance 1% from the sequence of the protein of interest using the free online program ExPASy, on the ExPASy website (www.expasy.org), the "ProtParam" button was selected and the sequence of the enzyme can then be input. The result was obtained by clicking "Compute parameters". The result from the computed parameters includes the molecular weight, amino acid composition, atomic composition, extinction coefficient, and the estimated half-life of the enzyme of interest.[27]

3.2.3 | Ellman's Activity Assay. The Ellman's Activity Assay is performed using the Agilent 8453 UV–Vis Spectrophotometer. A typical Ellman's assay contains of 5× LuxS Buffer, dH_2O, increasing concentrations of SRH, 1.6 μM Co-BsLuxS-HT, and 150 μM of DTNB (Ellman's Reagent). The contents are added into a quartz cuvette with a final volume of 1 mL. The UV–Vis spectrophotometer is set to measure the protein activity at 412 nm for a total of 204 seconds every 17 seconds.[3,5,7] The initial rate of each run from the increasing concentration of SRH is taken and graphed using GraphPad Prism. The concentrations of SRH substrate used to help determine the activity of the Co-BsLuxS-HT are 0.000 μM, 1.060 μM, 2.125 μM, 4.250 μM, 8.500 μM, 17.000 μM, 34.000 μM, and 68.000 μM. As the

concentration of SRH substrate is increased, it can be seen visually at the end of the kinetic assay that the solution contents turn more yellow, which is the product from the Ellman's reagent. The Ellman's assay of the Co-BsLuxS-HT reaction can be described by the initial binding reaction of the substrate (SRH) to the enzyme Co-BsLuxS-HT, which produces homocysteine and DPD at a 1:1 ratio. The homocysteine reacts with the Ellman's reagent (DTNB) to form the yellow product, 2-nitro-5-thiobenzoate, which is being measured at 412 nm using the Agilent 8453 UV–Vis Spectrophotometer.[3,5,20] The initial rates (AU/s) that are obtained from UV–Vis spectrophotometer are converted into specific activity (μmol/mg/min).[7] The specific activity is input onto GraphPad Prism in the y-axis in correlation to the increasing concentration of SRH, which is input into the x-axis. GraphPad Prism will automatically calculate the K_M, V_{max}, k_{cat}, and K_{cat}/K_M values from the graph. A sample graph from an initial assay of LuxS is included in Figure 3.2.3 for illustration.

A



B

K_M (μM)	6.1
V_{max} (μmoles/min)	310
k_{cat} (min^{-1})	3.9
k_{cat} / K_M (μM^{-1} min^{-1})	0.64

Figure 3.2.3 | Michaelis-Menten Graph. (A) Above is a Michaelis-Menten graph of Co-BsLuxS-HT, which shows the activity of the enzyme as the concentration of the substrate, *S*-ribosylhomocysteine (SRH), is increased. (B) Kinetics results from Ellman's assay of Co-BsLuxS-HT.

The Michaelis constant K_M describes the amount of substrate concentration needed for enzyme reaction to occur at half-maximal velocity. Therefore, if the K_M value is high, a higher concentration of substrate is needed for catalysis to proceed, which means that the substrate has low affinity for the enzyme and vice versa.[28] V_{max} is the maximal velocity in which the enzyme catalyzes the reaction. k_{cat} is often considered as the "turnover number", which measures the catalytic production of products. Finally, k_{cat}/K_M measures the catalytic efficiency of the enzyme reaction. The greater the k_{cat}/K_M value is, the faster the substrate is converted into products.[28]

3.3 | Application of Spectrophotometric Techniques to the Study of LuxS. With the use of the different types of spectrophotometers mentioned above, the LuxS enzyme concentration can first be quantified after purification, and then the activity of the LuxS enzyme determined. Using the NanoDrop spectrophotometer as well as performing the Bradford assay on a plate reader such as the PerkinElmer EnVision, the LuxS concentration can be determined and compared accordingly. Furthermore, once potential inhibitors are targeted, the UV–Vis diode array spectrophotometer can be used to test whether inhibition is occurring by performing an activity assay such as the Ellman's assay.

Chapter 4: Purification of LuxS and Optimization of Activity Assay

4.1 | LuxS Purification. The purification of an enzyme is an important process in biochemistry, through which impurities are removed so that the desired protein can be isolated and obtained. There are several different options for purifying a protein, such as separation by size, affinity, or physical/chemical properties. Thus, before the purification of LuxS can occur, it is important to know that background of the Co-BsLuxS-HT. Co-BsLuxS-HT was inserted into the pET-22b(+) vector, which has six histidines attached on the C-terminus end of the DNA sequence and has a ampicillin resistance marker. Once the DNA is inserted into the vector by cloning, the DNA vector can be transformed into an expression cell-line. The expression cell-line used is BL21[DE3] Gold, which is used to express the LuxS protein sequence.[10,11] Based on previous studies done for the protein purification of Co-BsLuxS-HT enzyme, the enzyme was isolated using an affinity column. The affinity column of choice used in purifying the Co-BsLuxS-HT enzyme is a cobalt column.[7]

4.1.1 | LuxS Overexpression and Cell Harvesting. In order to isolate the Co-BsLuxS-HT protein, the purification of Co-BsLuxS-HT begins by overexpressing the cells for three days, which allows for the minimization and control of potential contamination. The procedure used mirrors that reported in the literature.[7] Cells are grown by initially inoculating a 5 mL LB starter culture containing ampicillin[75] with the Co-BsLuxS-HT stock and incubating it for 16 h overnight at 37 ^{o}C. The cell line in which the LuxS sequence was inserted in contains an ampicillin resistance sequence that allows only those cells resistant to this antibiotic to grow in culture. The next day, a 1:1000 back-dilution was performed by adding 50 μL of the

starter culture into 50 mL of LuxS minimal media containing ampicillin[75] and shaking it for 16 h in the incubator at 37 ^{o}C. The following day, a 1:50 back-dilution was done by adding 10 mL of the 1:1000 back-dilution culture into 500 mL of LuxS minimal media containing ampicillin[75] (2 L total). The 2 L cultures were shaken in the 37 ^{o}C incubator for 2 h with its OD_{600} was measured every 30 min. The desired optical density of bacteria to be obtained is at 0.6, this is because 0.6 is where the exponential growth of bacteria lies (cell-doubling).[29] Once the optical density has reached ~ 0.6, the cultures were removed from the shaker to decrease the temperature of the shaker to 30 ^{o}C and adding 200 μM of cobalt chloride. Additional cobalt chloride was added to ensure that the LuxS proteins are enriched with cobalt as its metal cofactor so that when eluted, the proteins are purple. The cultures were then put back into the shaker for an additional 30 min before inducing with 100 μM IPTG for 16 h overnight at 30 ^{o}C. Induction occurs using IPTG because it is part of the pET expression system control in which IPTG acts as the analog of the lac repressor in cell line. The lac genes are responsible for the expression of proteins involved in the breakdown of lactose or in this case IPTG, which because of its presence initiates transcription of the lac genes. The cells were harvested the next day by centrifuging the cells down at 5,000 rpm for 20 min. The cells were collected and stored at –80 ^{o}C until the cells are ready to be lysed.[10,29]

Table 4.1.2 | Representative OD_{600} values for LuxS overexpression cultures.

Absorbance Time (started after 2 h of shaking)	OD_{600} (Flask 1)	OD_{600} (Flask 2)	OD_{600} (Flask 3)	OD_{600} (Flask 4)
12:40	0.17	0.21	0.19	0.15
1:10	0.26	0.30	0.28	0.24
1:40	0.35	0.30	0.38	0.34
2:10	0.47	0.56	0.51	0.44
2:40	0.64	0.74	0.69	0.60

4.1.2 | Cell Lysis. There are many different methods used to lyse bacterial cells such as by sonication, French press, or microfluidizers. The reason for wanting to lyse a bacterial cell is to release its fluid contents, also known as its lysate. The lysate contains the desired enzyme; in this case, the desired enzyme is Co-BsLuxS-HT. After the cell has been lysed and spun down again, protein purification can then be performed.[30] While the literature reports a sonication-based method for the lysis of *E. coli* BL21[DE3] cells, previous work from our laboratory suggested that alternate means of cell lysis were appropriate given the equipment available.[32]

4.1.2.1 | French Press. The instrument of choice for the lysis of the bacterial cells containing the Co-BsLuxS-HT enzyme is the French Press. The French Press uses pressure to disrupt cells. The French Press uses a manual pump to create pressure that pushes a piston within a cylinder that contains a suspension of cells, which is then forced through a needle valve. The pressure generated by the force is then able to disrupt the cell membranes, allowing the lysate to be released. Preparation of the Co-BsLuxS-HT to be lysed includes suspending the cells

into 35 mL lysis buffer, which is then added into the French Press cylinder. The piston is then inserted at the top for the French Press to begin. The cells were then lysed at 20,000 psi and the collected lysate was spun down at 5,000 rpm using the centrifuge for 20 min at 4 ^{o}C.

Figure 4.1.2.1 | French Press. Image of the French pressure cell used to obtain the Co-BsLuxS-HT lysate.

4.1.3 | Affinity Chromatography. Affinity chromatography is a purification method used to separate mixtures given highly specific interactions between analyte and the stationary phase of the chromatography column. In the case of the enzyme Co-BsLuxS-HT, six histidines are artificially included in its sequence. This "His-tag" has an affinity for metal ions, particularly nickel and cobalt. This affinity allows for Co-BsLuxS-HT to bind onto a cobalt affinity column and elute out only with excess amounts of the metal ion ligand, imidazole.

4.1.3.1 | Hand Column. Originally, the purification of the Co-BsLuxS-HT enzyme was done using a hand column. The hand column uses gravity to push out the buffers and protein from

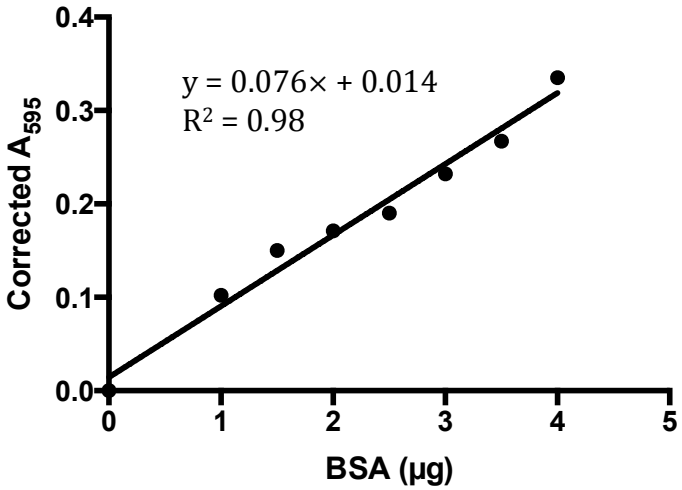
the column. Purification proceeds by adding 5 mL of TALON cobalt resin to the hand column; the Co-BsLuxS-HT enzyme was then purified by equilibrating the column with 3 column volumes of lysis buffer before adding the centrifuged lysate. The column was then washed with 35 mL of wash buffer, loaded with lysate, and eluted out in ~ 1 mL aliquots with 40 mL of elution buffer. Fractions containing LuxS were visually identified (purple coloration) and collected into a single sample for further analysis.

4.1.3.2 | ÄKTAprime Plus. The ÄKTAprime plus is an instrument that performs automated protein purification using pre-packed chromatography columns. For the purification of Co-BsLuxS-HT, the 5 mL HiTrap TALON crude was used to purify the proteins. The pre-packed column was attached to the ÄKTA and a pre-programmed protocol was chosen to run the purification process. The program chosen was "Affinity Purification any HiTrap". The purification proceeds almost the same way as the hand column, except that the program can control the flow-rate and pressure. Fractions containing LuxS were identified by the UV trace generated by the ÄKTA system. The concentration of protein in each individual fraction was determined by spectrophotometry (i.e., NanoDrop, see section 4.2.2) before combining.[10]

4.2 | Determination of Co-BsLuxS-HT Enzyme Concentration. It is imperative to have an accurate protein concentration when running any enzyme experiment, especially kinetic assays. Having a precise protein concentration allows for accurate comparability to published data. There are two possible spectrophotometric methods of finding the protein concentration of purified Co-BsLuxS-HT. The first is by a colorimetric assay called a Bradford assay and the second method is by using a NanoDrop spectrophotometer.

4.2.1 | Bradford Assay. Initially, the concentration of Co-BsLuxS-HT was determined by performing a Bradford assay. The Bradford assay is a colorimetric assay that is used to determine the protein concentration by the absorbance shift of the Coomassie Brilliant Blue G-250 dye (see Section 3.2.1). Here, the Bradford assay was performed using the EnVision spectrophotometer using 96-well plates. A standard curve was first created from a set of known BSA concentrations (Figure 4.2.1A). From this, it could then be determined what the unknown concentration of the Co-BsLuxS-HT protein is from the linear regression equation from the BSA standard set. In order to achieve an absorbance reading for LuxS that fell within the linear range of the standard curve, dilutions of 1:10, 1:20, 1:40, 1:80, and 1:160 were made to the collected LuxS fractions. The absorbance of the 1:80 dilution fell within the range of the standard curve graph and therefore was chosen as the concentration for the Co-BsLuxS-HT enzyme (Figure 4.2.1B).

A



B

LuxS Dilutions	Corrected Abs.	Concentration in mM
1:10 ELS1409	1.1	0.96
1:20 ELS1409	0.99	1.8
1:40 ELS1409	0.61	2.1
1:80 ELS1409	**0.34**	**2.3**
1:160 ELS1409	0.20	2.7

C

Absorbance: 0.34	Volume: 4 µL
Total Volume: 1400 µL	Factor: 80

Equation: $y = 0.076\times + 0.014$

Calculation:

(0.34 - 0.014) / 0.076 = 4.3 µg (amount of protein)

4.3 µg / 4 µL = 1.1 µg / µL

1.1 µg / µL × 80 = 85 µg / µL

(85 µg / µL ÷ 1000000) / 37000) × 1000000000 = 2.3 mM

Figure 4.2.1 | Bradford Assay. (A) Standard curve of Bradford assay using BSA standards. The standard curve was created in order to obtain the linear regression equation, which can be used to calculate the protein concentration of Co-BsLuxS-HT. (B) Dilutions of the Co-BsLuxS-HT was made in order to fit the standard curve, so that the protein concentration may be determined accurately. The absorbance of the dilution must fall within the standard curve and then the concentration can be calculated by solving for x. (C) Calculation of LuxS concentration from Bradford Assay.

4.2.2 | NanoDrop. The second method used to determine the concentration of Co-BsLuxS-HT is by using the NanoDrop spectrophotometer. The NanoDrop spectrophotometer method was introduced because of concentration discrepencies that arose in the Bradford assay. Using the NanoDrop, the concentration of Co-BsLuxS-HT can be directly determined with the use of its extinction coefficient (11,460 M^{-1} cm^{-1}) and the Co-BsLuxS-HT absorbance at 1%, which is 6.23, which were both aquired using the ProtParam tool from ExPASy by simply inputing the protein sequence of Co-BsLuxS-HT.[10] The NanoDrop requires only 2 μL of solution for the reading to proceed at absorbance of 280 nm. The conclusion to use the NanoDrop concentration over the Bradford assay was finalized by the similarity in data it obtained when running the Ellman's assay in comparison to literature reports.[7]

Table 4.2.2 | NanoDrop Concentration Data.

NanoDrop Concentration (mg/mL)	Calculated Concentration (mM)
6.2	1.2

4.3 | Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE). SDS-PAGE is a popular technique used in biochemistry and in other similar disciplines. The sodium dodecyl sulfate (SDS) is a detergent used to denature proteins so that all of the protein structure are linearized to the same shape and altered in the overall charge to be negative. The gel used is a polyacrylamide gel, which allows for the separation of proteins by size. When the gel is placed in an electric field, the proteins are then separated based on their size. The proteins travel by size towards the positive pole with smaller proteins

travelling faster than the larger proteins, which means that at the end of the run, the smaller proteins will be at the bottom of the gel.

4.3.1 | SDS-PAGE to Determine LuxS Purity. After the purificaiton of Co-BsLuxS-HT from the ÄKTAprime plus, an SDS-PAGE was run in order to check its purity. A pure Co-BsLuxS-HT protein will show a single band at ~18 kD on an SDS-PAGE gel. As mentioned earlier, the purity of the enzyme Co-BsLuxS-HT is of great signifance to the success of running kinetic assays. Therefore, before the Co-BsLuxS-HT enzyme is used in the Ellman's assay or Inhibition assay, its purity must always be checked using the SDS-PAGE after its protein concentration has been determined either by Bradford assay or NanoDrop spectrophotometer.

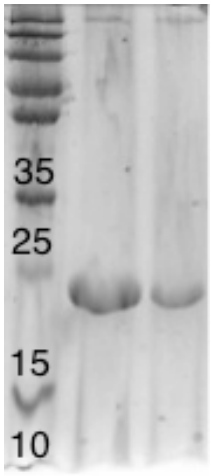


Figure 4.3.1 | SDS-PAGE. SDS-PAGE of Co-BsLuxS-HT from its purification using ÄKTAprime plus. The Co-BsLuxS-HT enzyme monomer is ~18 kD in size, which is confirmed by this SDS-PAGE analysis.

4.4 | Optimization of Ellman's Assay for LuxS Activity. The Ellman's assay is used for the quantification of thiols in a sample. *S*-D-Ribosyl-L-homocysteine (SRH) is the substrate that is converted into DPD and homocysteine (a thiol) by the enzyme Co-BsLuxS-HT. In the Ellman's assay, the homocysteine is then converted into a disulfide conjugate with DTNB, which releases the compound 2-nitro-5-thiobenzoate. 2-Nitro-5-thiobenzoate is a yellow product that is monitored at 412 nm. The conditions that are required for a typical Ellman's assay are 1.6 μM Co-BsLuxS-HT enzyme, 5× LuxS Buffer, DTNB, and increasing concentrations of SRH.[7,10] Initially, the Ellman's assay required troubleshooting because of the rapid conversion of the homocysteine into the 2-nitro-5-thiobenzoate (yellow product) that resulted in unacceptably high background. Therefore, changes were made to the preparatory stages of the Ellman's assay. Previously, a combined mixture of the 5× LuxS Buffer and DTNB were prepared accordingly to the amount that will be used for each run. The SRH was diluted to each specific concentration that was to be tested, while adding the Co-BsLuxS-HT protein last was to initiate the reaction for the assay. In protocol revision, it was decided that every component should be added individually for each assay run at each desired concentration. The irregular activity shown in the previous methodology could be due to the fact that SRH was diluted well prior to the assay, which sat on ice for quite some time before usage. The order that was best suited for the assay and gave the most optimal result in correlation to Pei *et al.*[7] was to initially add the 5× LuxS Buffer, desired concentration of SRH that was taken directly from stock (and not previously diluted), DTNB, and Co-BsLuxS-HT. By following this specific order, the assay did not initiate until the Co-BsLuxS-HT was added to the opposing side of the cuvette from the DTNB and mixed by turning the cuvette over twice before inserting it into the UV–Vis spectrophotometer for

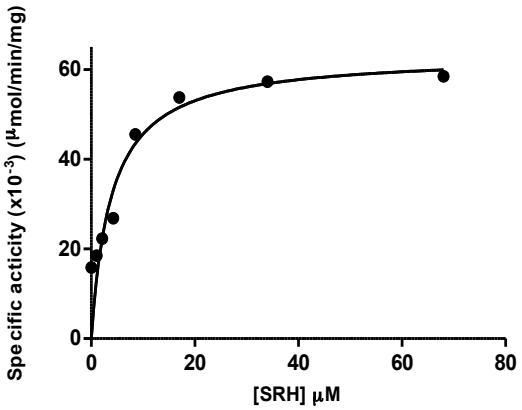
reading. (See Table 2.3.3 for the particular concentrations and volumes of each assay component used.)

4.4.1 | Ellman's Assay. The Ellman's Activity Assay is performed using the Agilent 8453 UV–Vis Spectrophotometer. A typical Ellman's assay contains of 5× LuxS Buffer, dH_2O, increasing concentrations of SRH, 1.6 μM Co-BsLuxS-HT, and 150 μM of DTNB (Ellman's Reagent). The contents are added into a quartz cuvette with a final volume of 1 mL. The UV–Vis spectrophotometer is set to measure the protein activity at 412 nm for a total of 204 seconds every 17 seconds. The initial rate of each run from the increasing concentration of SRH is taken and graphed using GraphPad Prism. The concentrations of SRH substrate used to help determine the activity of the Co-BsLuxS-HT are 0.000 μM, 1.060 μM, 2.125 μM, 4.250 μM, 8.500 μM, 17.000 μM, 34.000 μM, and 68.000 μM.[3,7,10] As the concentration of SRH substrate is increased, it can be seen visually at the end of the kinetic assay that the solution contents turn more yellow, which is the product from the Ellman's reagent. The Ellman's assay of the Co-BsLuxS-HT reaction can be described by the initial binding reaction of the substrate (SRH) to the enzyme Co-BsLuxS-HT, which produces homocysteine and DPD at a 1:1 ratio. The homocysteine reacts with the Ellman's reagent (DTNB) to form the yellow product, 2-nitro-5-thiobenzoate, which is being measured at 412 nm using the Agilent 8453 UV–Vis Spectrophotometer.[7] The initial rates (AU/s) that are obtained from UV–Vis spectrophotometer are converted into specific activity (μmol/mg/min).[1,3,7,12] (See Appendix D for Calculation). The specific activity is inputted onto GraphPad Prism in the y-axis in correlation to the increasing concentration of SRH, which is inputted into the x-axis. GraphPad Prism will automatically calculate the K_M, V_{max}, k_{cat}, and k_{cat}/K_m values from the graph. The Michaelis constant K_M describes the amount of substrate concentration needed

for enzyme reaction to occur. Therefore, if the K_M value is high, a higher concentration of substrate is needed for catalysis to proceed, which means that the substrate has low affinity for the enzyme and *vice versa*. V_{max} is the maximal velocity in which the enzyme catalyzes the reaction. k_{cat} is often considered as the "turnover number", which measures the catalytic production of products. Finally, k_{cat}/K_M measures the catalytic efficiency of the enzyme reaction. The greater the k_{cat}/K_M value is, the faster the substrate is converted into products.[7,14,28]

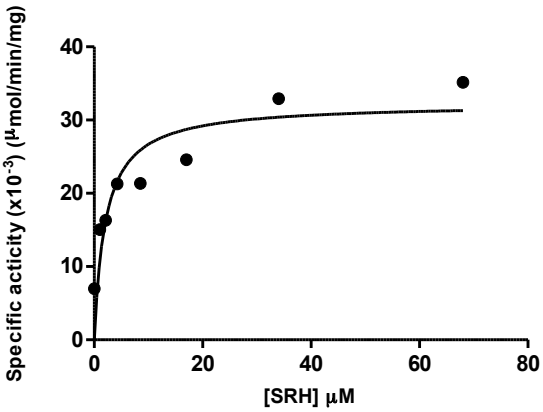
4.4.2 | Kinetic Comparison. The protein concentration results from the Bradford assay and NanoDrop spectrophotometer were compared by comparing the Ellman's assay results of each because of the disparity between the kinetics data obtained from the Bradford concentration and the reported literature values.[7] The NanoDrop spectrophotometer was therefore used to solve this variation issue since its concentration values seem to match the kinetics data and graph of the literature values more than the values that were aquired from the Bradford assay.

A (Nanodrop)



Assuming 1.2 mM protein concentration

B (Bradford Assay)



Assuming 2.40 mM protein concentration

C

Kinetic constants	Kinetics Data from Bradford Concentration	Kinetics Data from NanoDrop Concentration	Kinetics Data as reported in the literature[7]
V_{max} (µM/s)	63	32	Not provided
K_M (µM)	3.9 ± 0.94	2.1 ± 0.95	2.3 ± 0.5
k_{cat} (s^{-1})	0.79 ± 0.050	0.40 ± 0.040	0.04 ± 0.003

Figure 4.4.2 | Kinetic Comparisons. (A) Kinetic assay using the concentraion obtained from the nanodrop, which is 1.2 mM. Assay is done by taking the intial rate of each assay run of increasing concentration of SRH and plotting it using GraphPad Prism. (B) Kinetic assay of Co-BsLuxS-HT with its concentration obtained from the Bradford assay, which was calculated to be 2.40 mM. The graph was created by plotting the intial rates from each of the increasing concentrations of SRH onto GraphPad Prism. (C) Table of the kinetic values obtained from GraphPad Prism. Although the data do not match the literature precisely, the concentration obtained from the NanoDrop gave enzyme activity closer to the one in the literature.[7]

4.5 | Conclusion and Future Directions. The Ellman's Activity assay has been completely optimized with the use of the NanoDrop Spectrophotometer ND-1000 to obtain accurate protein concentration and optimized reagent preparation and order of addition. By

implementing the Michaelis–Menten equation, the activity of the Co-BsLuxS-HT enzyme can be determined from the K_M, V_{max}, k_{cat}, and k_{cat}/K_M values and compared to values reported in the literature. For future directions, it would be advantageous to run the Ellman's Activity assay using the PerkinElmer EnVision Multilabel Plate Reader so that multiple assays can be run at once and with significantly less material.

Chapter 5: Computational Screening for Potential Competitive Inhibitors of LuxS

Computational screening, or virtual screening, is a method used for the discovery of bioactive small molecules such as substrates in drug discovery. Screening can either be ligand-based or structure-based. For ligand-based screening, a model receptor is determined for sets of known ligands or structurally similar ligands by testing how well the ligands bind to the model receptor, whereas structure-based screening is involved in scoring a library of ligands that can differ from one another and determining the affinity of each ligand to the known receptor. Computational screening has been a key method for drug discovery within the science industry.[16,23] Many companies use computational chemists to help look for substrates that would competitively inhibit their target. Other times, substrates are designed to be part of a docking system as a potential substrate for an unknown target yet to be discovered. For this particular experiment, the purpose is to computationally screen for substrates using databases containing a variety of compounds that can potentially bind competitively to an enzyme active site (structure-based). Through the database, each substrate is docked onto the target and scored on how well it binds. Most computational screening methodologies require knowledge of Linux systems to focus on designing ligands for a specific target. Recently, screening has been made more accessible to the general scientific community since programs such as DOCK Blaster have made it user-friendly because screening can be completely automated.[15]

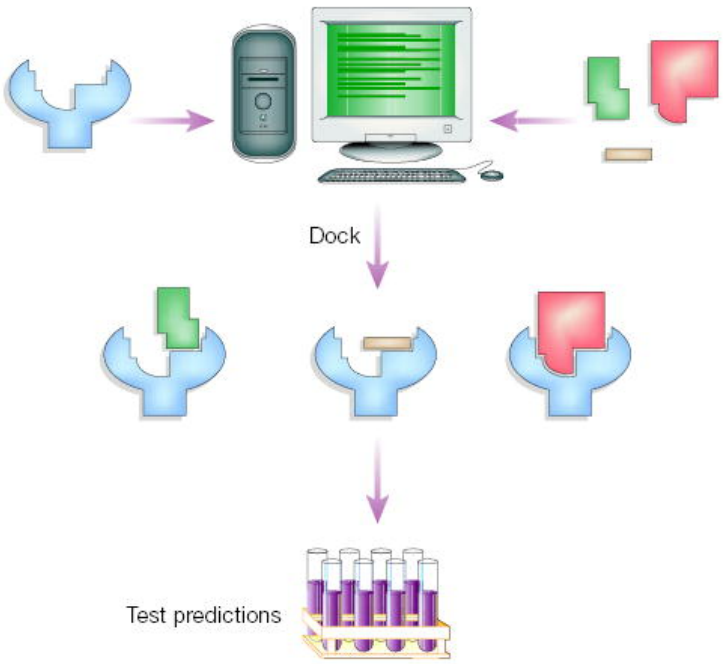


Figure 5 | DOCK Blaster Computational Screening Method. Overview of how computational screening programs such as DOCK Blaster perform. Target and substrates are docked together onto a computational program and scored based on binding specificity. Higher scoring substrates are tested using an assay to confirm the inhibition.[15,16]

5.1 | DOCK Blaster. The screening platform chosen for molecular screening of the LuxS enzyme for the discovery of more potential non-SRH analog inhibitors is DOCK Blaster. DOCK Blaster is a program created by the Shoichet lab from the University of California, San Francisco. It is available free of charge online, which allows easy access from any computer. DOCK Blaster can also be completely automated, therefore allowing for accessibility to the more general public without being an expert in computational chemistry. The ZINC database is linked to DOCK Blaster and acts as a self-analysis for the molecule docked. It re-docks the substrate (SRH) and docks decoys onto the active site in order to score the molecule on its pose-fidelity and (%) enrichment.[15,16]

5.1.1 | General Guidelines. For the docking of a set of small molecules to a protein target to proceed, a PDB code for the protein of interest is first required. The PDB codes of all known protein crystal structure can be retrieved from the RCSB Protein Data Bank (www.rcsb.org). The PDB structure is then initially "scored" by DOCK Blaster for its suitability for docking (Figure 5.1). A small molecule docking database from the DOCK Blaster website must be selected after the initial scoring of the molecule is complete. The chosen database (*e.g.*, KEGG or Cayman Chemical) will score every ligand it contains into the selected binding site of the protein and show its best 200 hits in the results page. From the results, ligands are chosen for *in vitro* biochemical validation based on score, availability for purchase, and affordability.

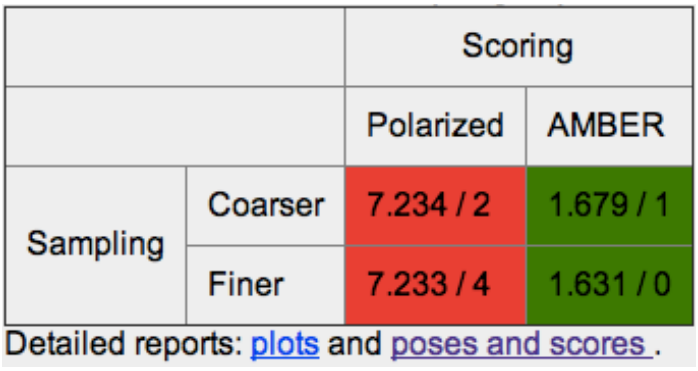


Figure 5.1.1 | DOCK Blaster Scoring. A sample calibration docking report of LuxS (PDB: 1JVI). The docking report shows the pose fidelity (Å, rmsd) and enrichment (% ranked) of the re-docked SRH (ligand) to the LuxS enzyme when compared to 100 decoys. It uses two sampling schemes (coarser and finer) to test different ligand orientations and two scoring schemes (Polarized and Normal / AMBER). Once SRH is properly scored, LuxS is docked with one of two databases for a chance to find a high-scoring ligand that will competitively bind in the LuxS active site.

5.1.2 | Closed-form SRH vs. Open-form SRH. *S*-D-Ribosyl-L-homocysteine (SRH) is the substrate that binds to the LuxS active site. It is known that SRH is in its linear aldose form when bound to the active LuxS. The compound "2-keto-SRH" is an intermediate between

SRH and DPD and is formed in part by the assisted ring opening by H_2O at the active site.[8] Since there is no existing protein crystal structure of the LuxS protein with the linear-SRH form bound in the active site, the 2-keto-SRH intermediate is used as a substitute. However, a recent investigation has shown that the α- or β- closed-form SRH has greater binding affinity (α-SRH more favorable with a binding energy of 47.5 kJ/mol less than the β-SRH) to the LuxS protein than the linear aldose form when performing computational screening. Hence, crystal structures containing both forms of SRH are used for the computational screening experiment.

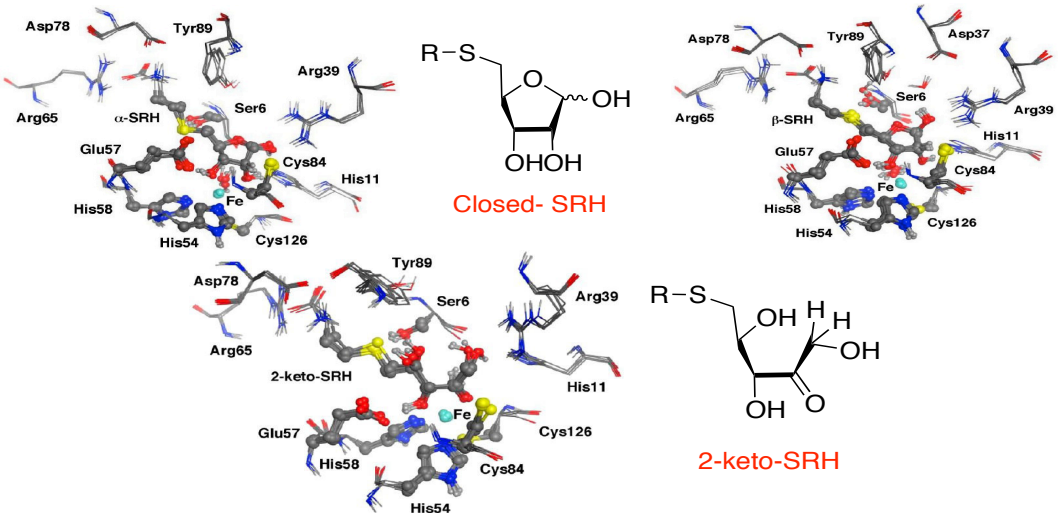
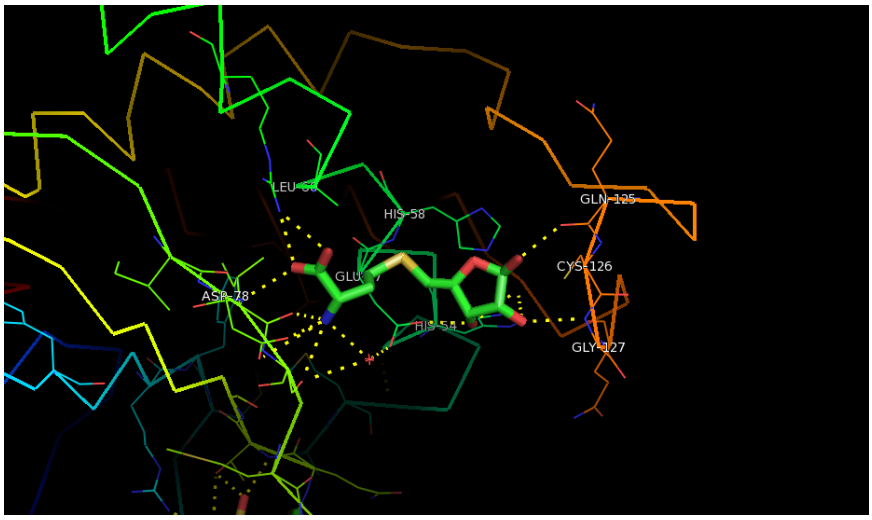


Figure 5.1.2 | Different Forms of SRH. According to Huang *et al*., SRH may bind to the LuxS enzyme when its ribosyl moiety is either in its α- or β-SRH form or as a linear aldose. The 2-keto-SRH is an intermediate in the conversion of SRH to the product DPD and is more favorable to bind in the LuxS active site than the closed-SRH forms.[8]

5.1.3 | RCSB Protein Data Bank (PDB). The Protein Data Bank contains all known protein crystal structures to date. It has information on three-dimensional structures of large biological molecules, including proteins. The Protein Data Bank, which can be found at www.rcsb.org, is freely available online to any users. Anyone can simply search for his or

her target by typing on the site's search bar. Here, typing "*S*-Ribosylhomocysteine" (SRH) in the search bar gives results that are separated based on the organism, X-ray resolution, release date, etc. In this case, the organism of focus is *Bacillus subtilis***.** From the options provided, the PDB codes chosen for the docking experiment portion are 1JVI and 1YCL. The 1JVI protein structure is from the organism *Bacillus subtilis* and has a resolution of 2.2 Å with a zinc ion at its active site, where the closed-form SRH is bound. The 1YCL protein crystal structure from the organism *Bacillus subtilis* has a 1.8 Å resolution and contains the 2-keto-SRH substrate, the intermediate of SRH and DPD. Since there is no crystal structure with the linear-SRH (open-form), the 2-keto-SRH is therefore used for computational screening because it is the closest in resemblance to the linear-SRH.

A



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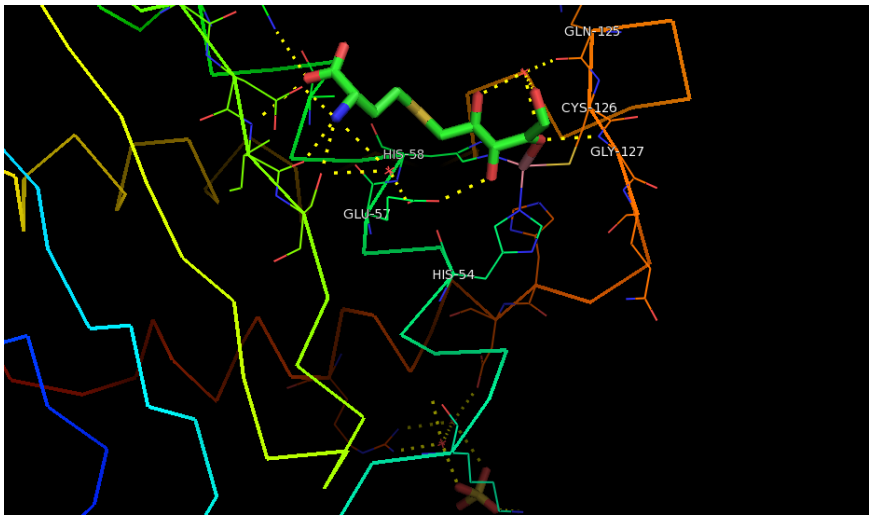


Figure 5.1.3 | PDB Codes. (A) Virtual image of the closed-form SRH in the active site of BsLuxS (PDB structure 1JVI) shown using PyMOL. (B) Virtual image of the SRH and DPD intermediate, 2-keto-SRH in the active site of BsLuxS (PDB structure 1YCL) shown.

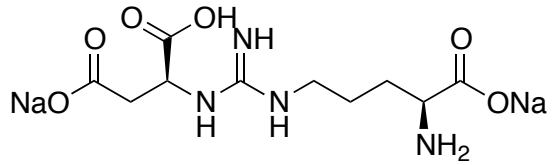
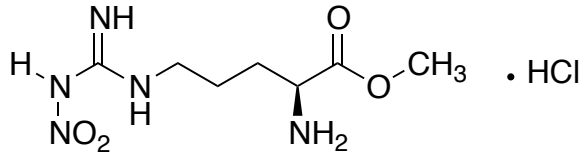
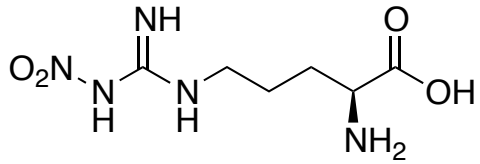
5.1.4 | Ligand Databases. For computational screening to proceed using the DOCK Blaster program, a database of virtual ligands must be chosen. Within the DOCK Blaster website, there are several databases already available for docking *in silico*; which means that no

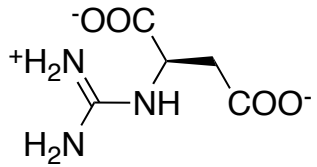
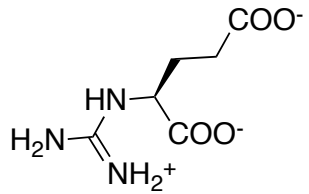
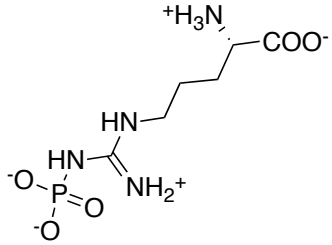
library needs to be created.[15] Three databases were chosen to run the experiment. The first database is KEGG. The reason why the KEGG database is chosen is because it contains the closed-SRH within its database, which allowed for a control experiment in the case of docking to the 1JVI LuxS crystal structure (see Figure 5.1.3A).[22] DOCK Blaster is able to dock the closed-SRH from the KEGG database back onto the LuxS active site, which demonstrated that the docking run is working correctly, while also providing ligands with better scores than the closed-SRH. Cayman Chemical is the second database where the LuxS enzyme is docked, this is due in part that the project goal is to find commercial substrates. Cayman Chemical is a widely known company to purchase substrates from and it guarantees us that the resulting substrates are truly purchasable.[31] Lastly, the FDA database was used for docking, however, to date the results from this database have not yet been tested. Nevertheless, the FDA database was chosen specifically for the purpose the substrates available within its database were already approved as potential drugs.[31]

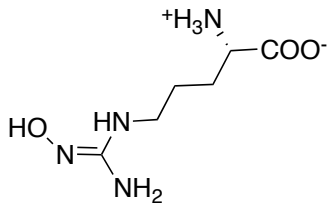
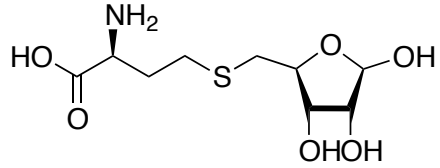
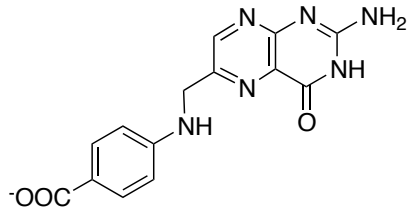
5.1.5 | Docking Visualization using PyMOL. PyMOL is a program that is widely used in the chemistry community, and it can be freely downloaded for academic purposes. PyMOL provides 3D images of proteins and, in this case, provides a visual image of whether the inhibitor has indeed bind to the active site *in silico*. The DOCK Blaster results can be directly opened and viewed through PyMOL, allowing us to see how every single docked result binds to the active site.

5.2 | Docking Results. From the docking of the 1JVI and 1YCL PDB codes, results were given from the ZINC database through the DOCK Blaster site. Some results from the two docked PDB codes overlapped, while others docked for one PDB code and did not dock for the other PDB code. The results are scored based on how well a ligand docks onto the LuxS active site compared to the original SRH substrate. The substrate results vary in structure. There are structures that contain halogens or phosphate groups that are known to be indicators for a good competitive inhibitor drug. The majority of the docking results were proven to be non-purchasable or were considered purchasable as "make on demand", which was considered inaccessible as well (see Appendix A). The affordability for those "hit" compounds that are purchasable such as argininosuccinate and L-NAME are priced at around twenty dollars.

Table 5.2 | 1JVI vs. 1YCL. Results obtained from the docking of both 1JVI and 1YCL PDB codes. Table shows the similarities and difference in substrate results obtained from both PDB codes.

Compound Name/ Zinc ID/ CAS # Chemical Structure	1JVI Rank / Score	1YCL Rank / Score
Argininosuccinate / 1529646 / CAS# 918149-29-8 	15/ –66	7 / –94
L-NAME/ 15987659 / CAS# 2149-70-4 	19/ –64	x
N-nitro-L-arginine/ 19796052 / CAS# 2149-70-4 	29/ –62	54 / –75

N-amido-L-aspartate / 4096031 / CAS# 6133-30-8 	35 / -62	156 / –67
(S)-(-)-2-Guanidinoglutaric Acid / 1529598 / CAS# 73477-53-9 	44 / –61	117 / –69
Phospho-L-arginine Trisodium Salt / 1530092 / CAS# 1189-11-3 	67 / –59	2 / –97

N^G-Hydroxy-L-arginine / 13558688 / CAS# 53598-01-9 	67 / –59	2 / –97
S-Ribosyl-L-homocysteine / 4096144 	126 / –55 (Re-docked)	x
Pteroic acid / 18182503 / CAS# 119-24-4 	x	104 / –70

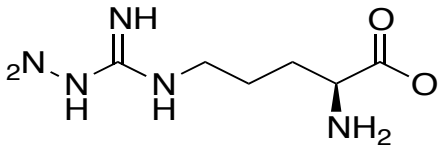
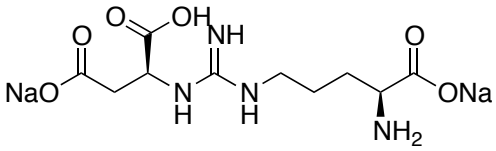
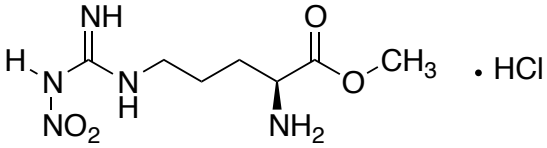
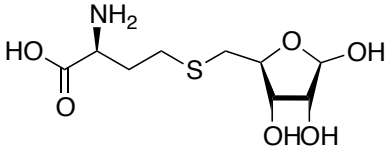
5.2.1 | KEGG Database Results. Two docking runs were performed on the 1JVI PDB codes in order to test whether there would be a difference in in the outcome of structures given. As can be seen in the two sections that follow, the scoring between the "Faster" and "Slower" run of DOCK Blaster onto the KEGG database differs in the way they are scored. This is due to the carefulness of scoring of each substrate and how it is bound to the active site. The faster a substrate is scored the less likely it could be scored appropriately; however, for the purpose of these initial investigations, the "Faster" scoring is sufficient in giving results. Running the slower run is less agreeable because of the amount of time it takes for docking to complete.[15] Further testing on the results from the slower run needs to be done in the future since majority of the results were never tested in order to obtain an accurate observation on whether the results given from the slower run are better. The only result from the slower run that was tested using the inhibition assay was Sulfasalazine, which later was proven to be an unreliable source since the substrate itself colored the solution yellow, confounding the Ellman's assay readout. Therefore, Sulfasalazine was not a suitable test subject for the comparison between the "Faster" and "Slower" run of the KEGG database. To date, all of the leading substrates that showed inhibition were results obtained from the faster run of the KEGG database

5.2.1.1 | Initial Results. For the initial run performed from query #72834 (PDB code 1JVI using faster scoring), the pose fidelity (Å, rmsd) and enrichment (% ranked) for the re-docking of SRH ranks at the very top with no other substrates ranking higher, as can be seen by the table result given by DOCK Blaster shown below. When the molecules from the KEGG database were docked into the 1JVI structure, SRH was identified as a result, ranking at 126. This not only means that the screening results are likely reliable, but also that 125

substrates identified *in silico* may potentially bind better than SRH to the LuxS active site. A full listing of these hit compounds is included in Appendix A. Interestingly, a number of these 125 "hit" compounds had structures similar to that of the amino acid arginine. These compounds and their screening scores are shown in the table below.

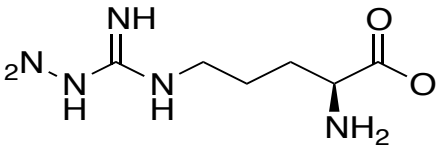
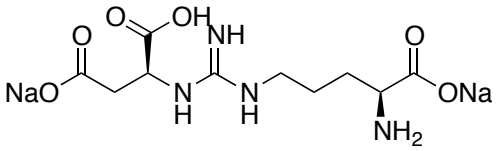
		Scoring	
		Polarized	AMBER
Sampling	Coarser	1.491 / 0	2.502 / 7
	Finer	1.265 / 0	1.51 / 6

DOCK Blaster > 1JVI < KEGG Database > Faster Run < 72834

Name of Compound	Compound Structure	Score (kcal/mol) / Rank
***N*-nitro-L-arginine**		−62/ 29
Argininosuccinate		−66/ 15
L-Nitroarginine methyl ester hydrochloride (L-NAME)		−64/ 19
***S*-Ribosylhomocysteine (SRH) - control**		−55/ 126

5.2.1.2 | Second-Round Results. The results obtained from the slower run of the 1JVI PDB code, Job I.D. #63945 did not score the SRH as a potential inhibitor. It gives higher negative scores than the faster run. At least one arginine-related compound was identified in this screen. However, to date only the substrate Sulfasalazine was ever tested from this run and it was judged to be a very invalid substrate to test because it turned the solution yellow, which interfered with the monitoring of the actual inhibition since the 2-nitro-5-thiobenzoate that is being monitored is yellow. Therefore, further testing needs to be conducted in order to determine whether results obtained from the slower run are more accurate compared to the faster run.

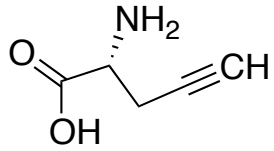
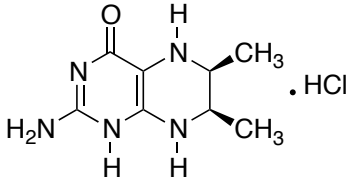
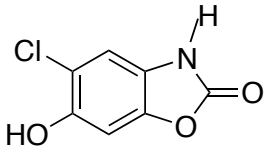
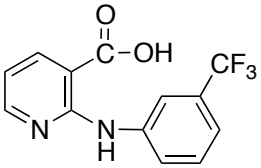
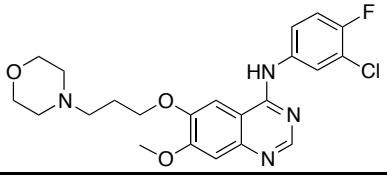
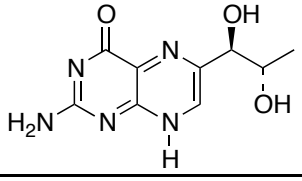
DOCK Blaster > 1JVI < KEGG Database > Slower Run < 63945

Name of Compound	Compound Structure	Score (kcal/mol) / Rank
2'-Deoxyguanosine-5'-monophosphoric acid disodium salt		–970/ 34
Sulfasalazine		–950 / 46

5.2.2 | Cayman Chemical Database Run Results. Selected DOCK Blaster results from the 1JVI PDB code run, query run #67436 using the Cayman Chemical database are shown below. Since SRH is a non-commercial compound, it is to be expected that it would not be within the Cayman Chemical database and therefore this positive control is not available. On a positive note, when docking through Cayman Chemical, the results given are known to be purchasable through the Cayman Chemical website. This screen gave a variety of new structures, many of which remain to be validated *in vitro*. (For the full collection of query #67436 hits, see Appendix A.)

Table 5.2.2

DOCK Blaster > 1JVI< Cayman Chemical > 67436

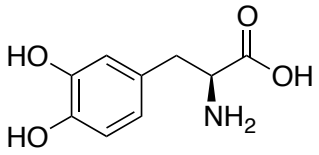
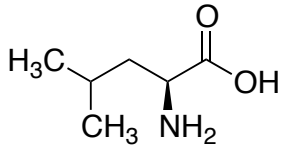
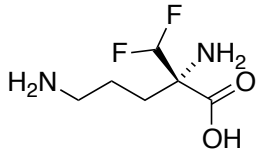
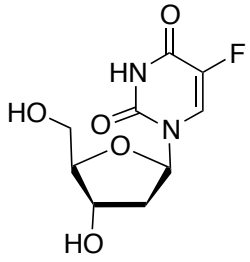
Name of Compound	Compound Structure	Score (kcal/mol) / Rank
D-Propargylglycine		–43 / 24
6,7-Dimethyltetrahydropterin (hydrochloride)		–42 / 28
6-Hydroxy Chlorzoxazone		–34 / 96
Niflumic Acid		–34/ 112
Gefitinib		–32/ 146
Biopterin		–47 / 11

5.2.3 | FDA Database Run Results. From the same DOCK Blaster 1JVI PDB code run result, query run # 63945, the FDA database was also run. The database was chosen for the purpose that substrates that were obtained from this database are FDA approved. Although

the FDA database was run, none of the substrates have yet been tested. This is due to results from previous structures that showed promise and therefore, more assays were done from substrates that were more similar to results (such as argininosuccinate and L-NAME).

Table 5.2.3

DOCK Blaster> 1JVI < FDA > 67436

Name of Compound	Compound Structure	Score (kcal/mol) / Rank
Levodopa		–50 / 6
L-Leucine		–50 / 5
Eflornithine Hydrochloride		–35 / 42
5-Fluoro-2'-deoxyuridine		–34 / 49

5.3 | Conclusion and Future Directions. The future goal for the docking portion of this project is to continue to dock the LuxS proteins (1JVI or 1YCL) using other molecule databases that could lead to more arginine-based substrates that were not initially discovered

in the KEGG and Cayman Chemical database as well as to test the inhibitors that were given as a result from the FDA database and other interesting candidates that have not yet been assayed. In the hopes that more promising leads come from DOCK Blaster, similar structure searches from inhibitors that showed promise from the Ellman's assay could be conducted to find a competitive inhibitor that would significantly be better than those that have already been reported.

Chapter 6: Selection and *in vitro* Testing of Potential Competitive Inhibitors of LuxS

Inhibition assays are common methods to observe whether a potential new inhibitor has successfully inhibited its target site in an enzyme of interest. There are different types of enzyme inhibition, such as uncompetitive inhibition, mixed inhibition, non-competitive inhibition, and competitive inhibition. In this project, the goal is to search for potential competitive inhibitors of the enzyme LuxS through computational screening. Selected "hit" results from *in silico* experiments (Chapter 5) were tested *in vitro* using a colorimetric assay called an Ellman's assay. It was expected that if a result from the computational screening were to competitively bind to the active site of the LuxS enzyme, then the activity of the enzyme would decrease and hence, less colored product is produced under Ellman's assay conditions.

6.1 | Competitive Inhibition. The goal of the project is to discover new potential competitive inhibitors of LuxS that are non-SRH analogs. Competitive inhibition is a type of inhibition in which an inhibitor binds to the same active site as an enzyme's substrate; in this case the substrate is SRH. Both the substrate and inhibitor cannot be bound to the enzyme's active site at the same time. Either the substrate or inhibitor (or neither) is bound to the active site. Competitive inhibition is reversible; however, in the case of a potent competitive inhibitor it would take high concentrations of substrate, beyond that typically available *in vivo*, to overcome the inhibition. In competitive inhibition, the maximum velocity (V_{max}) is

unchanged; however, since the substrate is less favorable to bind at the active site, the K_d will decrease and hence, K_M will increase.[14]

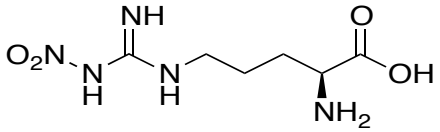
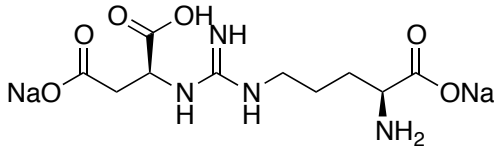
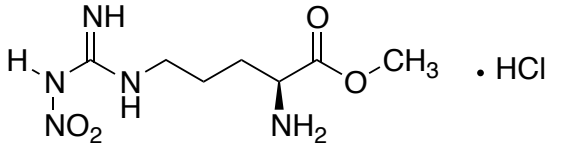
6.1.1 | General Guidelines for Inhibition Assays. The preparation of an inhibition assay includes a potential inhibitor that was a result from a reliable DOCK Blaster run (see Chapter 5). Again, the goal of the project is to search for purchasable potential competitive inhibitors that are non-SRH analogs. The assay also needs the 1.6 μM purified LuxS enzyme (see Chapter 4), 34 μM SRH substrate, 150 μM Ellman's reagent (DTNB), 5× LuxS Buffer, and deionized water. Competitive inhibition is dose-dependent, and therefore increasing amounts of inhibitor are added to each of a series of cuvettes during the assay. The inhibitor concentrations used for the assays are 68 μM, 136 μM, 204 μM, 272 μM, and 340 μM.

6.2 | DOCK Blaster Hit Categories. For each docking of the *B. subtilis* LuxS 1JVI and 1YCL PDB code, 200 potential inhibitors result. The 1JVI PDB code was docked with the KEGG database, the Cayman Chemical database, and FDA database, whereas 1YCL was only docked with the KEGG database (see Chapter 5). Below is a table that shows the compounds that were results from DOCK Blaster that have, to date, been tested *in vitro*. The docking of the 1JVI and 1YCL PDB code gave results that showed promise such as argininosuccinate and L-NAME. Initially, *N*-nitro-L-arginine and argininosuccinate were tested for inhibition. The results from these assays can be seen below. *N*-Nitro-L-arginine shows minimal inhibition; however, argininosuccinate produced data that showed more pronounced inhibition in initial trials. Hence, another arginine-based structure, L-NAME, which was a lower-ranking DOCK Blaster hit, was also tested. The IC_{50} from the L-NAME

Ellman's Assay run showed that it was even more competitive than argininosuccinate. From these results, it was decided that since both substrates showed inhibition from the Ellman's assay, a similarity structure search of arginine would be thoroughly investigated and if permitted, tested.

6.2.1 | Arginine-Based Structures. DOCK Blaster results that were tested and showed similarity in its foundation as the amino acid, Arginine are shown below. The scoring of these arginine-based structures are relevant in that L-NAME showed the most significant inhibition when compared to argininosuccinate and when argininosuccinate is compared to *N*-nitro-L-arginine.

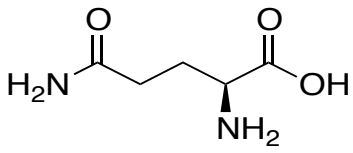
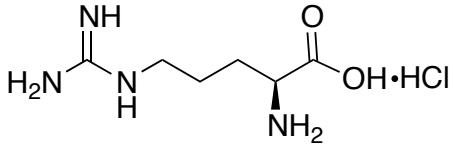
DOCK Blaster> Results

Name of Compound	Compound Structure	Score (kcal/mol) / Rank
***N*-Nitro-L-arginine**		–62 / 35
Argininosuccinate		–60 / 44
L-Nitroarginine methyl ester hydrochloride (L-NAME)		–59 / 60

6.2.1.1 | Similarity Structure Searches. From the DOCK Blaster results that gave promising inhibition results, a similar structure search was done on *N*-nitro-L- arginine and argininosuccinate. The similar structure searches were done using Sigma-Aldrich structure search and SciFinder. Those that do not have a similar structure score were chosen logically based on its similarity in structure to arginine. These results can be seen in the tables below.

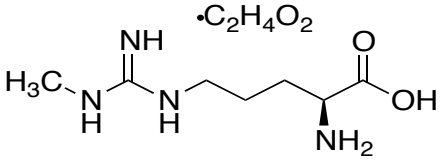
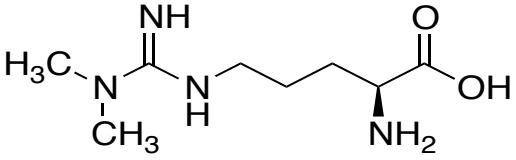
6.2.1.1.1 | SciFinder Similarity Searches.

Similar Structure Search> SciFinder< Results

Name of Compound	Compound Structure	Similarity Score
L-Glutamine		83
L-Arginine hydrochloride		82

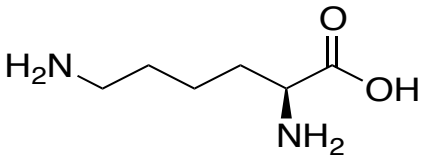
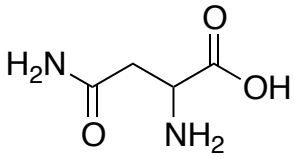
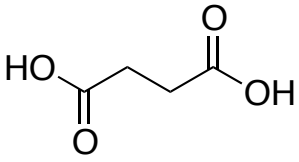
6.2.1.1.2 | Sigma-Aldrich Similarity Searches.

Similar Structure Search> Sigma-Aldrich< Results

Name of Compound	Compound Structure	Similarity Score
N^G-Methyl-L-arginine acetate salt		50
N^G, N^G-Dimethylarginine dihydrochloride		50

6.2.1.1.3 | Other Structural Types. Other amino acids that may be the closest in structure similarity to Arginine are also investigated to see whether others will show greater inhibition than Arginine.

Similar Structure Search> Arginine-based< Results

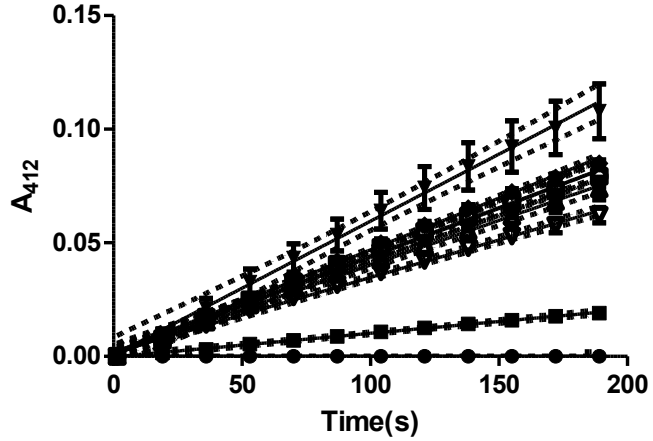
Name of Compound	Compound Structure	Similarity Score
L-Lysine		--
D,L-Asparagine		--
Succinic Acid		--

6.3 | Assays of Selected Compounds. The inhibitors that have been tested thus far are *N*-nitro-L-arginine, argininosuccinate, L-NAME, succinic acid, L-glutamine, L-arginine hydrochloride, N^G-methyl-L-arginine acetate salt, N^G, N^G- dimethylarginine dihydrochloride, D,L- asparagine, and L-lysine. The inhibitors were chosen based on its availability for purchase, affordability, and its similarity to the arginine structure. The inhibitors were results of screening *in silico* and / or of similarity structure searches.

6.3.1 | Argininosuccinate and Similar Structures. From the initial findings of the Ellman's assay for argininosuccinate, which gave an IC_{50} of 490 μM for this *in silico* screening "hit", it was determined that the focus of the project will continue to look into arginine-based compounds. Therefore, structures similar to it were also tested. N^G-Methyl-L-arginine acetate salt and N^G, N^G- dimethylarginine dihydrochloride are two of a few structures that are given from the similar structure search of argininosuccinate through Sigma–Aldrich and that were actually assayed to determine if inhibition is occurring.

6.3.1.1 | Argininosuccinate. The first promising result that came from DOCK Blaster was argininosuccinate. Argininosuccinate was dissolved to a final stock concentration of 680 μM with 5× LuxS Buffer and deionized water. As described in section 2.4.2, the cuvette contained 5× LuxS Buffer, deionized water, 34 μM SRH, 1.6 μM Co-BsLuxS-HT enzyme, DTNB, and increasing concentrations of argininosuccinate. The LuxS inhibition assay of argininosuccinate was measured at 412 nm at increasing inhibitor concentrations using 68 μM, 136 μM, 204 μM, 272 μM, and 340 μM of argininosuccinate. The assay was done in triplicate and graphed using GraphPad Prism. Below shows the results in slopes of the linear regression fits of each concentration of inhibitor used in the assay as well as the standard error. In all cases, the y-intercept of the linear regression approximates zero.

A

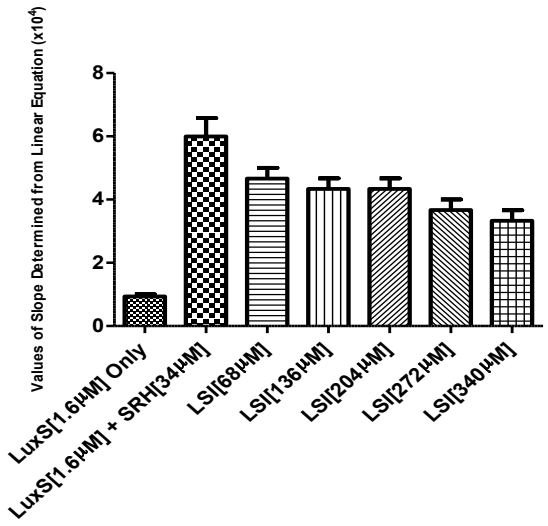


B

	LuxS [1.6 μM] Only	SRH [34 μM] Only	LuxS [1.6 μM] + SRH [34 μM]
Slope	1.0 (± 0.01) × 10^{-4}	7.5 (± 3.0) × 10^{-6}	5.9 (± 0.11) × 10^{-4}

	LSI [68 μM]	LSI [136 μM]	LSI [204 μM]	LSI [272 μM]	LSI [340 μM]
Slope	4.5 (± 0.067) × 10^{-4}	4.3 (± 0.077) × 10^{-4}	4.2 (± 0.092) × 10^{-4}	3.9 (± 0.050) × 10^{-4}	3.3 (± 0.095) × 10^{-6}

C

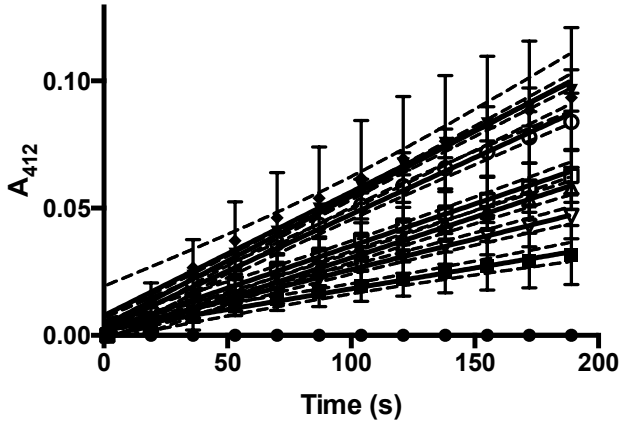


	LuxS [1.6 μM] Only	LuxS [1.6 μM] + SRH [34 μM]	LSI [68 μM]	LSI [136 μM]	LSI [204 μM]	LSI [272 μM]	LSI [340 μM]
Standard Error	0.067	0.58	0.33	0.33	0.33	0.33	0.33

Figure 6.3.1.1 | Argininosuccinate Inhibition Assay. Dose-dependent inhibition trials for argininosuccinate. (A) Average of 3 LuxS inhibition assay runs using argininosuccinate as the possible inhibitor. The error bars given on the graph are in standard error of 3 trials. (Legend: LSI = LuxS + SRH + Inhibitor , closed circle = Buffer Blank, closed square = LuxS[1.6 μM] only, closed triangle = SRH[34 μM] only, closed inverted triangle = LuxS[1.6 μM] + SRH[34 μM], closed diamond = LSI[68 μM], open circle = LSI[136 μM], open square = [204 μM], open triangle = LSI[272 μM], and open inverted triangle = LSI[340 μM]) (B) Shows the best-fit line for each subject using argininosuccinate as the inhibitor. C) Slopes from each LuxS inhibition run used to compare the activity under different concentrations of succinic acid. The column is produced from the average slope of each subject from all 3 trials. Error bars that are on the graph are the standard error of 3 trials for each subject.

6.3.1.2 | N^G-Methyl-L-arginine acetate salt. N^G-Methyl-L-arginine acetate salt (L-NMMA) was a Sigma–Aldrich result from a similarity structure search using argininosuccinate as the initial structure. N^G-Methyl-L-arginine acetate salt was dissolved with 5× LuxS buffer to achieve a 1 M stock concentration. A typical LuxS inhibition assay for N^G-Methyl-L-arginine acetate salt consist of 5× LuxS Buffer, deionized water, 34 μM SRH, 1.6 μM Co-BsLuxS-HT enzyme, Ellman's reagent (DTNB), and increasing concentrations of inhibitor. Concentrations of the inhibitor tested are 68 μM, 136 μM, 204 μM, 272 μM, and 340 μM and measured at 412 nm. The results were then graphed on GraphPad Prism.

A

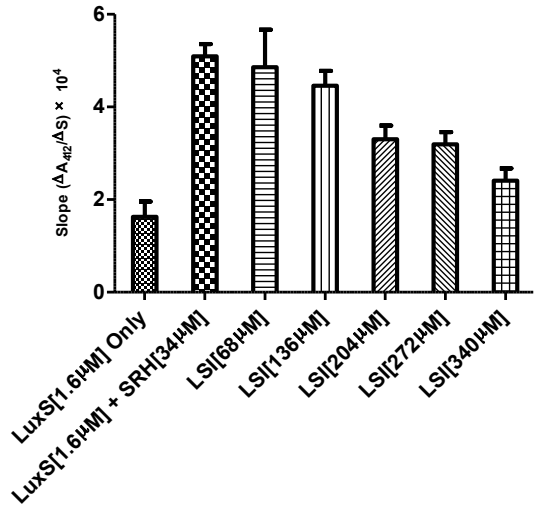


B

	LuxS [1.6 μM] Only	SRH [34 μM] Only	LuxS [1.6 μM] + SRH [34 μM]
Slope	$1.6 (\pm 0.049) \times 10^{-4}$	$-1.7 (\pm 0.56) \times 10^{-5}$	$5.1 \pm (0.11) \times 10^{-4}$

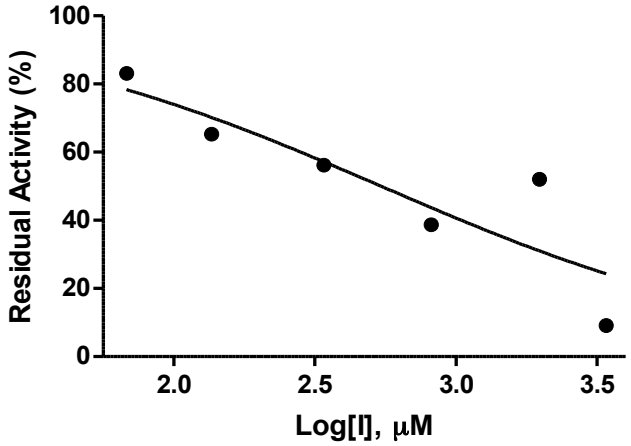
	LSI [68 μM]	LSI [136 μM]	LSI [204 μM]	LSI [272 μM]	LSI [340 μM]
Slope	$4.9 (\pm 0.22) \times 10^{-4}$	$4.5 (\pm 0.11) \times 10^{-4}$	$3.3 (\pm 0.061) \times 10^{-4}$	$3.2 (\pm 0.042) \times 10^{-4}$	$2.4 (\pm 0.48) \times 10^{-4}$

C



	LuxS [1.6 μM] Only	**LuxS [1.6 μM] + SRH [34 μM]**	**LSI [68 μM]**	**LSI [136 μM]**	**LSI [204 μM]**	**LSI [272 μM]**	**LSI [340 μM]**
Standard Error	0.33	0.27	0.81	0.32	0.30	0.26	0.27

D

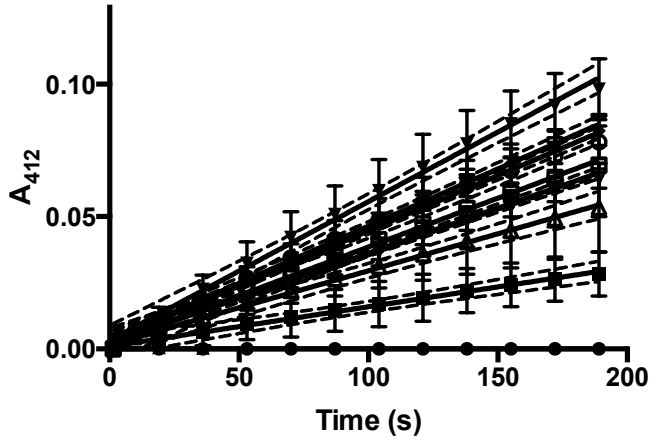


IC_{50} [μM]	$LogIC_{50}$ [μM]
540	2.7

Figure 6.3.1.2 | N^G-Methyl-L-arginine acetate salt (L-NMMA) Inhibtion Assay. To determine whether N^G-methyl-L-arginine acetate salt is a competitive inhibitor of LuxS by performing dose-dependent inhibition assays. (A) Graph of the average of (3) LuxS inhibition assay runs using N^G-methyl-L-arginine acetate salt as the possible inhibitor. The error bars given on the graph are in standard error of (3) trials. (Legend: LSI = LuxS + SRH + Inhibitor, closed circle = Buffer Blank, closed square = LuxS[1.6 μM] only, closed triangle = SRH[34 μM] only, closed inverted triangle = LuxS[1.6 μM] + SRH[34 μM], closed diamond = LSI[68 μM], open circle = LSI[136 μM], open square = [204 μM], open triangle = LSI[272 μM], and open inverted triangle = LSI[340 μM]) (B) Shows the best-fit line for each subject using N^G-methyl-L-arginine acetate salt as the inhibitor. C) Compares the activity of the enzyme by using the slopes obtained from each LuxS inhibition run, which uses different concentrations of N^G-methyl-L-arginine acetate salt. D) The IC_{50} graph of N^G-methyl-L-arginine acetate salt. The column is produced from the average slope of each point from all 3 trials.

6.3.1.3 | N^G, N^G- Dimethylarginine Dihydrochloride. N^G, N^G- dimethylarginine dihydrochloride was another Sigma–Aldrich result from a similarity structure search using argininosuccinate as the desired structure. N^G, N^G- Dimethylarginine dihydrochloride was dissolved with 5× LuxS buffer to achieve a 1 M stock concentration. A typical LuxS inhibition assay for N^G, N^G- dimethylarginine dihydrochloride consist of 5× LuxS Buffer, deionized water, 34 μM SRH, 1.6 μM Co-BsLuxS-HT enzyme, Ellman's reagent (DTNB), and increasing concentrations of inhibitor. Concentrations of the inhibitor tested are 68 μM, 136 μM, 204 μM, 272 μM, and 340 μM and measured at 412 nm. The results were then graphed on GraphPad Prism.

A

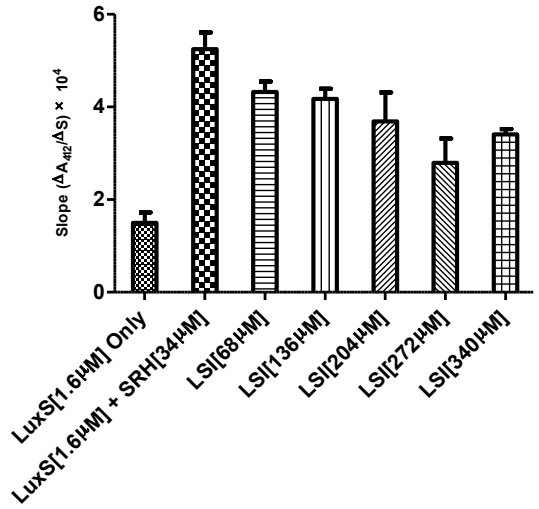


B

	LuxS [1.6 μM] Only	SRH [34 μM] Only	LuxS [1.6 μM] + SRH [34 μM]
Slope	$1.5 (\pm 0.030) \times 10^{-4}$	$-1.7 (\pm 0.29) \times 10^{-5}$	$5.2 (\pm 0.12) \times 10^{-4}$

	LSI [68 μM]	LSI [136 μM]	LSI [204 μM]	LSI [272 μM]	LSI [340 μM]
Slope	$4.3 (\pm 0.0093) \times 10^{-4}$	$4.2 (\pm 0.12) \times 10^{-4}$	$3.7 (\pm 0.0067) \times 10^{-4}$	$2.8 (\pm 0.004) \times 10^{-4}$	$3.4 (\pm 0.0059) \times 10^{-4}$

C

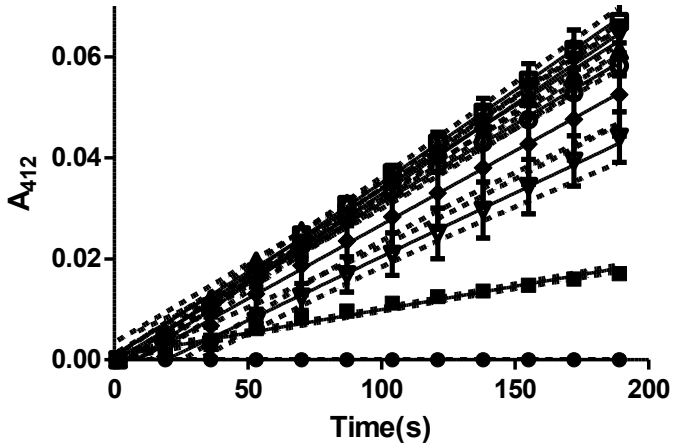


	LuxS [1.6 μM] Only	LuxS [1.6 μM] + SRH [34 μM]	LSI [68 μM]	LSI [136 μM]	LSI [204 μM]	LSI [272 μM]	LSI [340 μM]
Standard Error	0.22	0.36	0.23	0.22	0.62	0.53	0.11

Figure 6.3.1.3 | N^G, N^G- Dimethylarginine Dihydrochloride Inhibition Assay. Dose-dependent inhibition trials for N^G, N^G- dimethylarginine dihydrochloride. (A) Average of 3 LuxS inhibition assay runs using N^G, N^G- dimethylarginine dihydrochloride as the possible inhibitor. The error bars given on the graph are in standard error of 3 trials. (Legend: LSI = LuxS + SRH + Inhibitor , closed circle = Buffer Blank, closed square = LuxS[1.6 μM] only, closed triangle = SRH[34 μM] only, closed inverted triangle = LuxS[1.6 μM] + SRH[34 μM], closed diamond = LSI[68 μM], open circle = LSI[136 μM], open square = [204 μM], open triangle = LSI[272 μM], and open inverted triangle = LSI[340 μM]) (B) Shows the best-fit line for each subject using N^G, N^G- dimethylarginine dihydrochloride as the inhibitor. C) Slopes from each LuxS inhibition run used to compare the activity under different concentrations of N^G, N^G- dimethylarginine dihydrochloride. The column is produced from the average slope of each subject from all 3 trials. Error bars that are on the graph are the standard error of 3 trials for each subject.

6.3.1.4 | Succinic Acid. Succinic acid was a potential inhibitor that was investigated based upon its similarity in structure to argininosuccinate (representing approximately one-half of the molecular structure). Succinic acid was dissolved with 5× LuxS Buffer and deionized water. The LuxS inhibition assay for succinic acid includes 5× LuxS Buffer, deionized water, 34 μM SRH, 1.6 μM Co-BsLuxS-HT enzyme, Ellman's reagent (DTNB), and increasing concentrations of inhibitor. Concentrations of the inhibitor tested are 68 μM, 136 μM, 204 μM, 272 μM, and 340 μM and measured at 412 nm. The results were then graphed on GraphPad Prism.

A

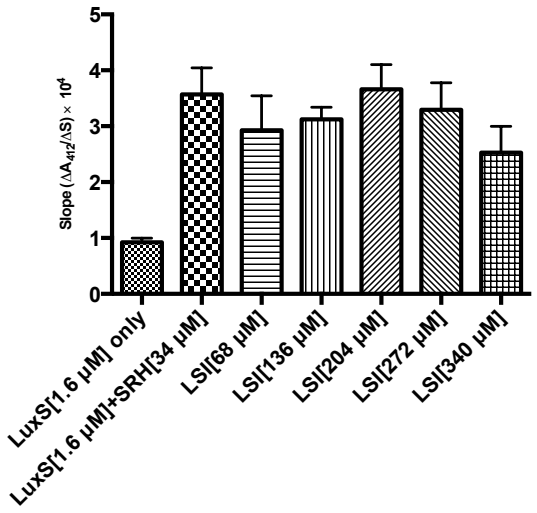


B

	LuxS [1.6 μM] Only	SRH [34 μM] Only	LuxS [1.6 μM] + SRH [34 μM]
Slope	$9.2 (\pm 0.29) \times 10^{-5}$	$-3.3 (\pm 0.64) \times 10^{-5}$	$3.6 (\pm 0.093) \times 10^{-4}$

	LSI [68 μM]	LSI [136 μM]	LSI [204 μM]	LSI [272 μM]	LSI [340 μM]
Slope	$2.9 (\pm 0.27) \times 10^{-4}$	$3.1 (\pm 0.060) \times 10^{-4}$	$3.7 (\pm 0.095) \times 10^{-4}$	$3.3 (\pm 0.11) \times 10^{-4}$	$2.5 (\pm 1.7) \times 10^{-4}$

C

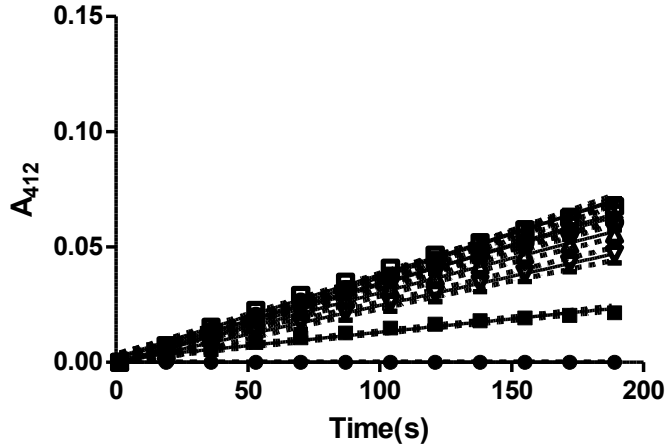


	LuxS [1.6 μM] Only	LuxS [1.6 μM] + SRH [34 μM]	LSI [68 μM]	LSI [136 μM]	LSI [204 μM]	LSI [272 μM]	LSI [340 μM]
Standard Error	0.043	0.28	0.36	0.12	0.26	0.28	0.27

Figure 6.3.1.4 | Succinic Acid Inhibition Assay. Dose-dependent inhibition trials for succinic acid. (A) Average of 3 LuxS inhibition assay runs using succinic acid as the possible inhibitor. The error bars given on the graph are in standard error of 3 trials. (Legend: LSI = LuxS + SRH + Inhibitor , closed circle = Buffer Blank, closed square = LuxS[1.6 μM] only, closed triangle = SRH[34 μM] only, closed inverted triangle = LuxS[1.6 μM] + SRH[34 μM], closed diamond = LSI[68 μM], open circle = LSI[136 μM], open square = [204 μM], open triangle = LSI[272 μM], and open inverted triangle = LSI[340 μM]) (B) Shows the best-fit line for each subject using succinic acid as the inhibitor. C) Slopes from each LuxS inhibition run used to compare the activity under different concentrations of succinic acid. The column is produced from the average slope of each subject from all 3 trials. Error bars that are on the graph are the standard error of 3 trials for each subject.

6.3.1.5| L-Arginine hydrochloride. L-Arginine hydrochloride was a SciFinder structure search result using argininosuccinate as the model of the type of structure desired (representing approximately one-half of the molecular structure). L-Arginine hydrochloride was dissolved with 5× LuxS Buffer and deionized water. A typical LuxS Inhibition assay included 5× LuxS Buffer, deionized water, 34 μM SRH, 1.6 μM Co-BsLuxS-HT enzyme, Ellman's reagent (DTNB), and increasing concentrations of inhibitor. Concentrations of the inhibitor tested are 68 μM, 136 μM, 204 μM, 272 μM, and 340 μM and measured at 412 nm. The results were then graphed on GraphPad Prism.

A

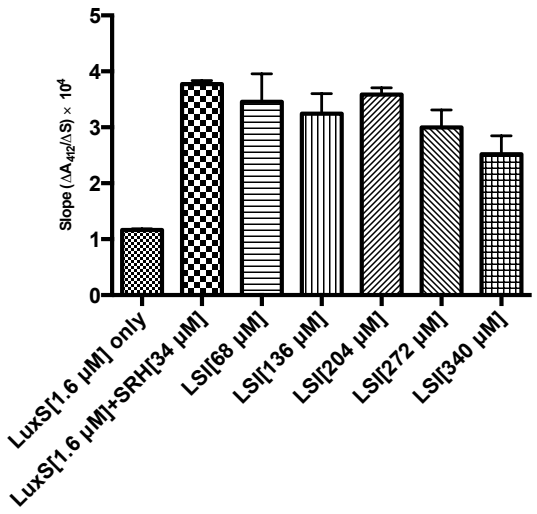


B

	LuxS [1.6 μM] Only	SRH [34 μM] Only	LuxS [1.6 μM] + SRH [34 μM]
Slope	$1.2 (\pm 0.047) \times 10^{-4}$	$-8.8 (\pm 7.2) \times 10^{-6}$	$3.8 (\pm 0.10) \times 10^{-4}$

	LSI [68 μM]	LSI [136 μM]	LSI [204 μM]	LSI [272 μM]	LSI [340 μM]
Slope	$3.5 (\pm 0.14) \times 10^{-4}$	$3.2 (\pm 0.086) \times 10^{-4}$	$3.6 (\pm 0.056) \times 10^{-4}$	$3.0 (\pm 0.12) \times 10^{-4}$	$2.5 (\pm 0.12) \times 10^{-4}$

C



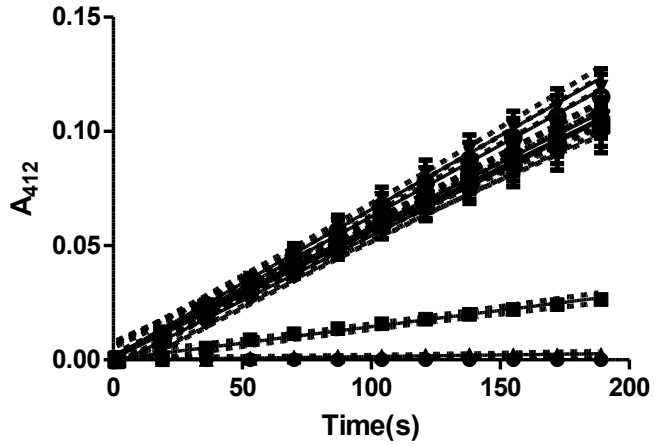
	LuxS [1.6 μM] Only	LuxS [1.6 μM] + SRH [34 μM]	LSI [68 μM]	LSI [136 μM]	LSI [204 μM]	LSI [272 μM]	LSI [340 μM]
Standard Error	0.013	0.037	0.29	0.21	0.068	0.18	0.19

Figure 6.3.1.5 | L-Arginine hydrochloride Inhibition Assay. Dose-dependent inhibition trials for L-arginine hydrochloride. (A) Average of 3 LuxS inhibition assay runs using L-arginine hydrochloride as the possible inhibitor. The error bars given on the graph are in standard error of 3 trials. (Legend: LSI = LuxS + SRH + Inhibitor , closed circle = Buffer Blank, closed square = LuxS[1.6 μM] only, closed triangle = SRH[34 μM] only, closed inverted triangle = LuxS[1.6 μM] + SRH[34 μM], closed diamond = LSI[68 μM], open circle = LSI[136 μM], open square = [204 μM], open triangle = LSI[272 μM], and open inverted triangle = LSI[340 μM]) (B) Shows the best-fit line for each subject using L-arginine hydrochloride as the inhibitor. C) Slopes from each LuxS inhibition run used to compare the activity under different concentrations of L-arginine hydrochloride. The column is produced from the average slope of each subject from all 3 trials. Error bars that are on the graph are the standard error of 3 trials for each subject.

6.3.2 | Other Arginine-Based Structures. Other arginine-based structures were also included among the results from *in silico* docking experiments using DOCK Blaster. One of the initial results was *N*-nitro-L-arginine, which showed minimal inhibition in initial trials. However, because argininosuccinate showed promising results, the related compound L-NAME was also tested for inhibition.

6.3.2.1 | *N*-Nitro-L-arginine. *N*-Nitro-L-arginine is the first inhibitor tested using the LuxS Inhibition Assay. *N*-Nitro-L-arginine was dissolved with 0.1 M HCl to a final stock concentration of 680 μM. A typical mixture for a LuxS Inhibition assay includes 5× LuxS Buffer, deionized water, 34 μM SRH, 1.6 μM wild-type Co-BsLuxS-HT enzyme, Ellman's reagent (DTNB), and increasing concentration of inhibitor. The assay is done in triplicate for reproducibility and reliability purposes. The concentrations of *N*-nitro-L-arginine tested are 68 μM, 136 μM, 204 μM, 272 μM, and 340 μM. The dose-dependent inhibition was measured at 412 nm and the results are graphed using GraphPad Prism.

A

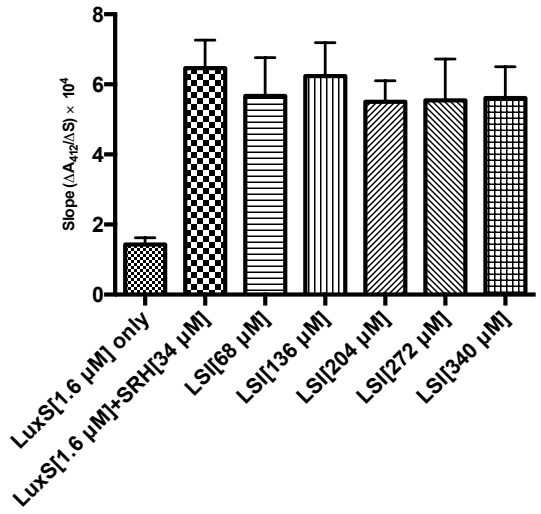


B

	LuxS[1.6μM] Only	SRH[34μM] Only	LuxS[1.6μM]+SRH[34μM]
Slope	$1.4\ (\pm\ 0.010) \times 10^{-4}$	$1.4\ (\pm\ 0.46) \times 10^{-5}$	$6.5\ (\pm\ 0.22) \times 10^{-4}$

	LSI[68μM]	LSI[136μM]	LSI[204μM]	LSI[272μM]	LSI[340μM]
Slope	$5.7\ (\pm\ 0.22) \times 10^{-4}$	$6.2\ (\pm\ 0.25) \times 10^{-4}$	$5.5\ (\pm\ 0.16) \times 10^{-4}$	$5.5\ (\pm\ 0.32) \times 10^{-4}$	$5.6\ (\pm\ 0.23) \times 10^{-4}$

C

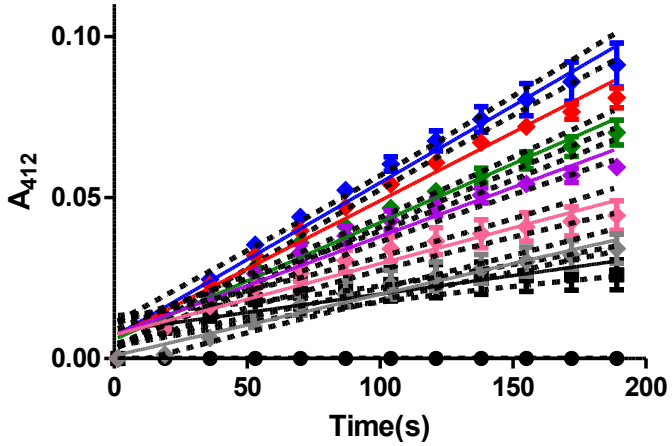


	LuxS [1.6 μM] Only	LuxS [1.6 μM] + SRH [34 μM]	LSI [68 μM]	LSI [136 μM]	LSI [204 μM]	LSI [272 μM]	LSI [340 μM]
Standard Error	0.11	0.46	0.63	0.55	0.34	0.68	0.52

Figure 6.3.2.1 | *N*-Nitro-L-arginine Inhibtion Assay. Dose-dependent inhibition trials for *N*-nitro-L-arginine. (A) Average of 3 LuxS inhibition assay runs using *N*-nitro-L-arginine as the possible inhibitor. The error bars given on the graph are in standard error of 3 trials. (Legend: LSI = LuxS + SRH + Inhibitor , closed circle = Buffer Blank, closed square = LuxS[1.6 μM] only, closed triangle = SRH[34 μM] only, closed inverted triangle = LuxS[1.6 μM] + SRH[34 μM], closed diamond = LSI[68 μM], open circle = LSI[136 μM], open square = [204 μM], open triangle = LSI[272 μM], and open inverted triangle = LSI[340 μM]) (B) Shows the best-fit line for each subject using *N*-nitro-L-arginine as the inhibitor. C) Slopes from each LuxS inhibition run used to compare the activity under different concentrations of *N*-nitro-L-arginine. The column is produced from the average slope of each subject from all 3 trials. Error bars that are on the graph are the standard error of 3 trials for each subject.

6.3.2.2 | L-Nitroarginine methyl ester hydrochloride (L-NAME). L-NAME was a DOCK Blaster result from the docking of the 1JVI PDB code, as well as a similarity structure result using argininosuccinate as the structure target. L-NAME was dissolved with 5× LuxS buffer to achieve a 1 M stock concentration. A typical LuxS inhibition assay for L-NAME consisted of 5× LuxS Buffer, deionized water, 34 μM SRH, 1.6 μM Co-BsLuxS-HT enzyme, Ellman's reagent (DTNB), and increasing concentrations of L-NAME. Concentrations of the inhibitor tested are 68 μM, 136 μM, 204 μM, 272 μM, and 340 μM and measured at 412 nm. The results were then graphed on GraphPad Prism.

A

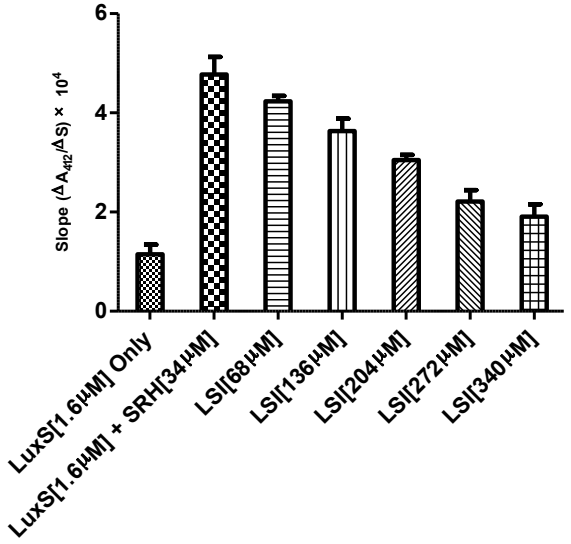


B

	LuxS [1.6 μM] Only	SRH [34 μM] Only	LuxS [1.6 μM] + SRH [34 μM]
Slope	1.1 (± 0.21) $\times 10^{-4}$	–1.6 (± 1.8)[a] $\times 10^{-5}$	4.8 (± 0.18) $\times 10^{-4}$

	LSI [68 μM]	LSI [136 μM]	LSI [204 μM]	LSI [272 μM]	LSI [340 μM]
Slope	4.2 (± 0.12) $\times 10^{-4}$	3.6 (± 0.13) $\times 10^{-4}$	3.0 (± 0.14) $\times 10^{-4}$	2.2 (± 0.18) $\times 10^{-4}$	1.9 (± 0.16) $\times 10^{-4}$

C



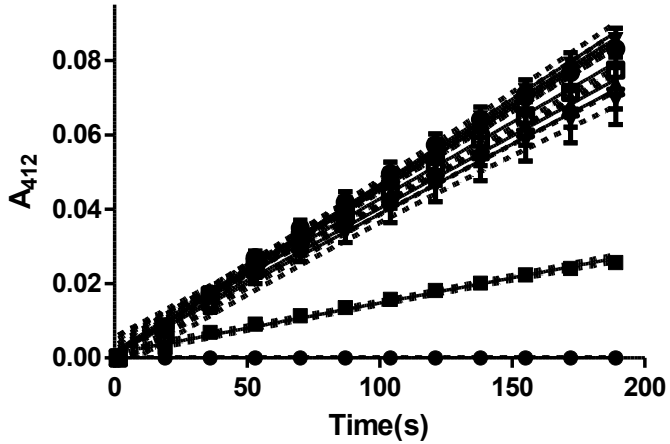
	LuxS [1.6 μM] Only	LuxS [1.6 μM] + SRH [34 μM]	LSI [68 μM]	LSI [136 μM]	LSI [204 μM]	LSI [272 μM]	LSI [340 μM]
Standard Error	0.19	0.35	0.11	0.25	0.11	0.23	0.25

Figure 6.3.2.2 | L-Nitroarginine methyl ester hydrochloride (L-NAME) Inhibition Assay. Dose-dependent inhibition trials for L-NAME. (A) Average of 3 LuxS inhibition assay runs using L-NAME as the possible inhibitor. The error bars given on the graph are in standard error of 3 trials. (Legend: LSI = LuxS + SRH + Inhibitor , closed circle = Buffer Blank, closed square = LuxS[1.6 μM] only, closed triangle = SRH[34 μM] only, closed inverted triangle = LuxS[1.6 μM] + SRH[34 μM], closed diamond = LSI[68 μM], open circle = LSI[136 μM], open square = [204 μM], open triangle = LSI[272 μM], and open inverted triangle = LSI[340 μM]) (B) Shows the best-fit line for each subject using L-NAME as the inhibitor. C) Slopes from each LuxS inhibition run used to compare the activity under different concentrations of L-NAME. The column is produced from the average slope of each subject from all 3 trials. Error bars that are on the graph are the standard error of 3 trials for each subject. [a]This high error value is attributed to the inherent uncertainty in fitting what is essentially noise when slope ≈ 0.

6.3.3 | Other Amino Acids. Based on the results that were being obtained from the arginine-based substrates, it was decided that it would of great interest to examine other amino acids. This is to make certain that arginine is the best amino acid to use as a base for drug design for the inhibition of the LuxS enzyme. The other amino acids that were examined were L-glutamine, L-lysine monohydrochloride, and DL-asparagine. Below are their Ellman's assay graphs to determine whether they achieved inhibition of the LuxS enzyme.

6.3.3.1 | L-Glutamine. L-Glutamine was a potential inhibitor that was chosen and investigated based on its similarity in structure to L-arginine hydrochloride. L-glutamine was dissolved with 5× LuxS Buffer and deionized water. The LuxS inhibition assay for L-glutamine includes 5× LuxS Buffer, deionized water, 34 μM SRH, 1.6 μM Co-BsLuxS-HT enzyme, Ellman's reagent (DTNB), and increasing concentrations of inhibitor. Concentrations of the inhibitor tested are 68 μM, 136 μM, 204 μM, 272 μM, and 340 μM and measured at 412 nm. The results were then graphed on GraphPad Prism.

A

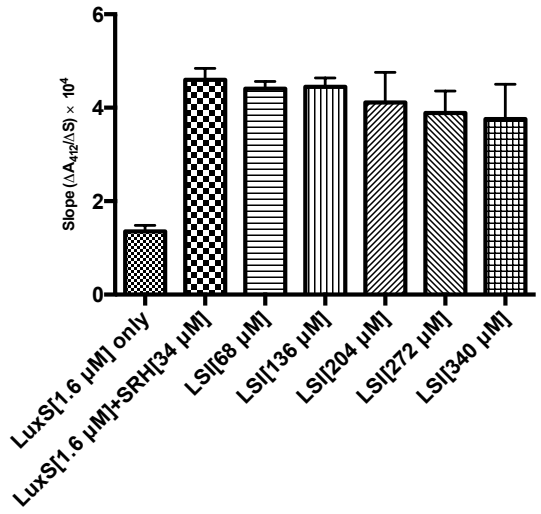


B

	LuxS [1.6 μM] Only	SRH [34 μM] Only	LuxS [1.6 μM] + SRH [34 μM]
Slope	$1.4 (\pm 0.041) \times 10^{-4}$	$-3.9 (\pm 0.94) \times 10^{-5}$	$4.6 (\pm 0.11) \times 10^{-4}$

	LSI [68 μM]	LSI [136 μM]	LSI [204 μM]	LSI [272 μM]	LSI [340 μM]
Slope	$4.4 (\pm 0.053) \times 10^{-4}$	$4.5 (\pm 0.066) \times 10^{-4}$	$4.1 (\pm 0.17) \times 10^{-4}$	$3.9 (\pm 0.14) \times 10^{-4}$	$3.8 (\pm 0.20) \times 10^{-4}$

C

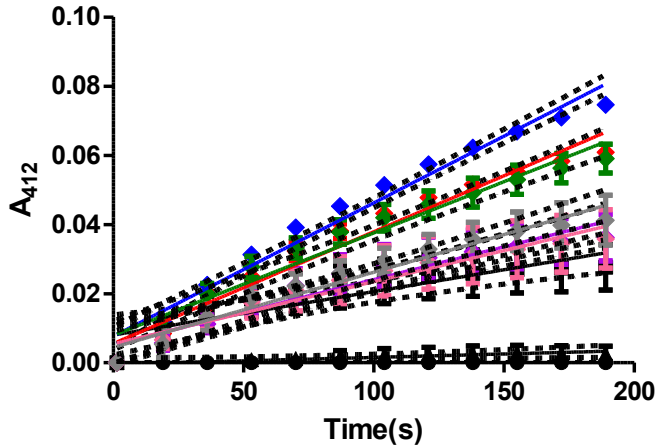


	LuxS [1.6 μM] Only	LuxS [1.6 μM] + SRH [34 μM]	LSI [68 μM]	LSI [136 μM]	LSI [204 μM]	LSI [272 μM]	LSI [340 μM]
Standard Error	0.075	0.14	0.089	0.11	0.37	0.27	0.43

Figure 6.3.3.1 | L-Glutamine Inhibition Assay. Dose-dependent inhibition trials for L-glutamine. (A) Average of 3 LuxS inhibition assay runs using L-glutamine as the possible inhibitor. The error bars given on the graph are in standard error of 3 trials. (Legend: LSI = LuxS + SRH + Inhibitor , closed circle = Buffer Blank, closed square = LuxS[1.6 μM] only, closed triangle = SRH[34 μM] only, closed inverted triangle = LuxS[1.6 μM] + SRH[34 μM], closed diamond = LSI[68 μM], open circle = LSI[136 μM], open square = [204 μM], open triangle = LSI[272 μM], and open inverted triangle = LSI[340 μM]) (B) Shows the best-fit line for each subject using L-glutamine as the inhibitor. C) Slopes from each LuxS inhibition run used to compare the activity under different concentrations of L-glutamine. The column is produced from the average slope of each subject from all 3 trials. Error bars that are on the graph are the standard error of 3 trials for each subject.

6.3.3.2 | L-Lysine monohydrochloride. L-Lysine monohydrochloride was a structure tested based on its similarity to L-arginine hydrochloride, in the hopes that it would prove that the arginine-based structures are unique and important to the inhibition of Co-BsLuxS-HT enzyme. L-lysine monohydrochloride was dissolved with 5× LuxS buffer to achieve a 1 M stock concentration. A typical LuxS inhibition assay for L-lysine monohydrochloride consisted of 5× LuxS Buffer, deionized water, 34 μM SRH, 1.6 μM Co-BsLuxS-HT enzyme, Ellman's reagent (DTNB), and increasing concentrations of L-lysine monohydrochloride. Concentrations of the inhibitor tested are 68 μM, 136 μM, 204 μM, 272 μM, and 340 μM and measured at 412 nm. The results were then graphed on GraphPad Prism.

A

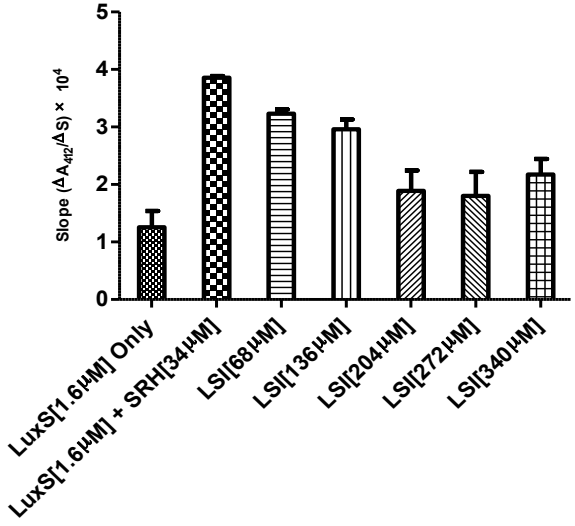


B

	LuxS [1.6 μM] Only	SRH [34 μM] Only	LuxS [1.6 μM] + SRH [34 μM]
Slope	1.3 (± 0.26) × 10^{-4}	2.0 (± 0.78) × 10^{-5}	3.9 (± 0.12) × 10^{-4}

	LSI [68 μM]	LSI [136 μM]	LSI [204 μM]	LSI [272 μM]	LSI [340 μM]
Slope	3.2 (± 0.14) × 10^{-4}	3.0 (± 0.18) × 10^{-4}	1.9 (± 0.23) × 10^{-4}	1.8 (± 0.28) × 10^{-4}	2.2 (± 0.20) × 10^{-4}

C

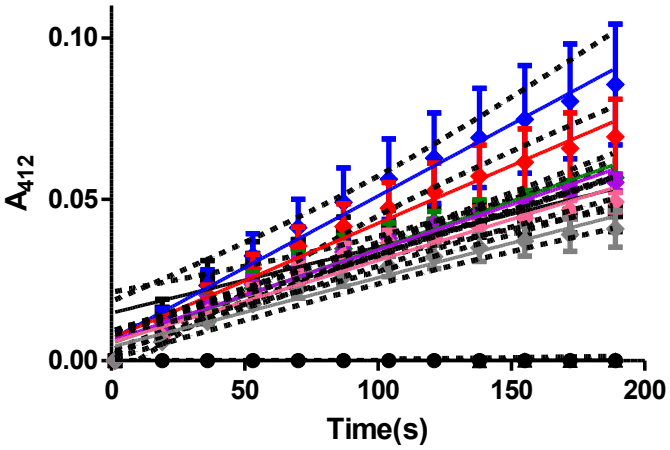


	LuxS [1.6 μM] Only	LuxS [1.6 μM] + SRH [34 μM]	LSI [68 μM]	LSI [136 μM]	LSI [204 μM]	LSI [272 μM]	LSI [340 μM]
Standard Error	0.28	0.030	0.071	0.17	0.35	0.42	0.27

Figure 6.3.3.2| L-Lysine monohydrochloride Inhibition Assay. Dose-dependent inhibition trials for L-lysine monohydrochloride. (A) Average of 3 LuxS inhibition assay runs using L-lysine monohydrochloride as the possible inhibitor. The error bars given on the graph are in standard error of 3 trials. (Legend: LSI = LuxS + SRH + Inhibitor , closed circle = Buffer Blank, closed square = LuxS[1.6 μM] only, closed triangle = SRH[34 μM] only, closed inverted triangle = LuxS[1.6 μM] + SRH[34 μM], closed diamond = LSI[68 μM], open circle = LSI[136 μM], open square = [204 μM], open triangle = LSI[272 μM], and open inverted triangle = LSI[340 μM]) (B) Shows the best-fit line for each subject using L-lysine monohydrochloride as the inhibitor. C) Slopes from each LuxS inhibition run used to compare the activity under different concentrations of L-lysine monohydrochloride. The column is produced from the average slope of each subject from all 3 trials. Error bars that are on the graph are the standard error of 3 trials for each subject.

6.3.3.3 | DL-Asparagine. DL-Asparagine was another structure that was evaluated based on its similarity to L-arginine hydrochloride. This was in the hopes that it would prove that arginine-based structures are the fundamental basis for the discovery of a potential competitive inhibitor for the Co-BsLuxS-HT enzyme. DL-Asparagine was dissolved with 5× LuxS buffer to achieve a 1 M stock concentration. A typical LuxS inhibition assay for DL-asparagine consist of 5× LuxS Buffer, deionized water, 34 μM SRH, 1.6 μM Co-BsLuxS-HT enzyme, Ellman's reagent (DTNB), and increasing concentrations of DL-asparagine. Concentrations of the inhibitor tested are 68 μM, 136 μM, 204 μM, 272 μM, and 340 μM and measured at 412 nm. The results were then graphed on GraphPad Prism.

A

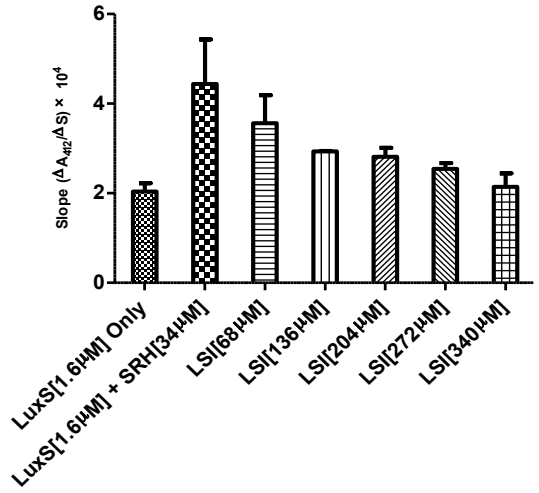


B

	LuxS [1.6 µM] Only	SRH [34 µM] Only	LuxS [1.6 µM] + SRH [34 µM]
Slope	2.0 (± 0.28) × 10^{-4}	3.3 (± 5.3)[a] × 10^{-6}	4.4 (± 0.52) × 10^{-4}

	LSI [68 µM]	LSI [136 µM]	LSI [204 µM]	LSI [272 µM]	LSI [340 µM]
Slope	3.6 (± 0.33) × 10^{-4}	2.9 (± 0.16) × 10^{-4}	2.8 (± 0.13) × 10^{-4}	2.5 (± 0.15) × 10^{-4}	2.1 (± 0.16) × 10^{-4}

C

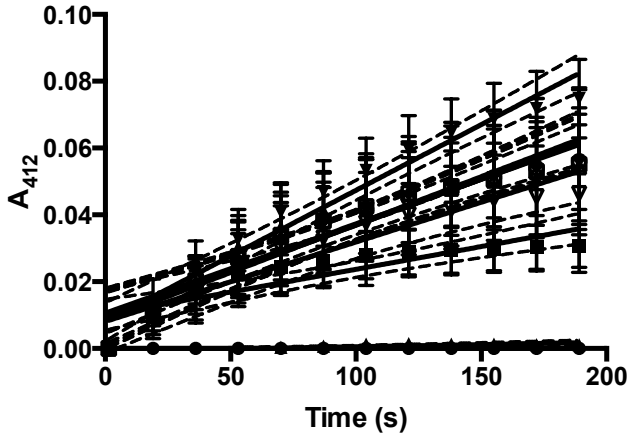


	LuxS [1.6 μM] Only	LuxS [1.6 μM] + SRH [34 μM]	LSI [68 μM]	LSI [136 μM]	LSI [204 μM]	LSI [272 μM]	LSI [340 μM]
Standard Error	0.18	1.0	0.63	0.012	0.20	0.13	0.30

Figure 6.3.3.3 | DL-Asparagine Inhibition Assay. Dose-dependent inhibition trials for DL-asparagine. (A) Average of 3 LuxS inhibition assay runs using DL-asparagine as the possible inhibitor. The error bars given on the graph are in standard error of 3 trials. (Legend: LSI = LuxS + SRH + Inhibitor , closed circle = Buffer Blank, closed square = LuxS[1.6 μM] only, closed triangle = SRH[34 μM] only, closed inverted triangle = LuxS[1.6 μM] + SRH[34 μM], closed diamond = LSI[68 μM], open circle = LSI[136 μM], open square = [204 μM], open triangle = LSI[272 μM], and open inverted triangle = LSI[340 μM]) (B) Shows the best-fit line for each subject using DL-asparagine as the inhibitor. C) Slopes from each LuxS inhibition run used to compare the activity under different concentrations of DL-asparagine. The column is produced from the average slope of each subject from all 3 trials. Error bars that are on the graph are the standard error of 3 trials for each subject. [a]This high error value is attributed to the inherent uncertainty in fitting what is essentially noise when slope ≈ 0.

6.3.4 | Other Structural Types. As mentioned previously, the 1JVI PDB code was docked onto the KEGG database a second time using the slower run. One of the results given from the slower run, Job I.D. #63945 docking, was Sulfasalazine. Sulfasalazine was dissolved with 5× LuxS buffer to achieve a 1 M stock concentration. The components in a typical LuxS inhibition assay for sulfasalazine includes 5× LuxS Buffer, deionized water, 34 μM SRH, 1.6 μM Co-BsLuxS-HT enzyme, Ellman's reagent (DTNB), and increasing concentrations of inhibitor. Concentrations of the inhibitor tested are 68 μM, 136 μM, 204 μM, 272 μM, and 340 μM and measured at 412 nm. The results were then graphed on GraphPad Prism, however, it was concluded from the very beginning that sulfasalazine was not an ideal inhibitor candidate. This is because sulfasalazine turned out to be yellow in color as a solid and then turned yellow in solution.

A

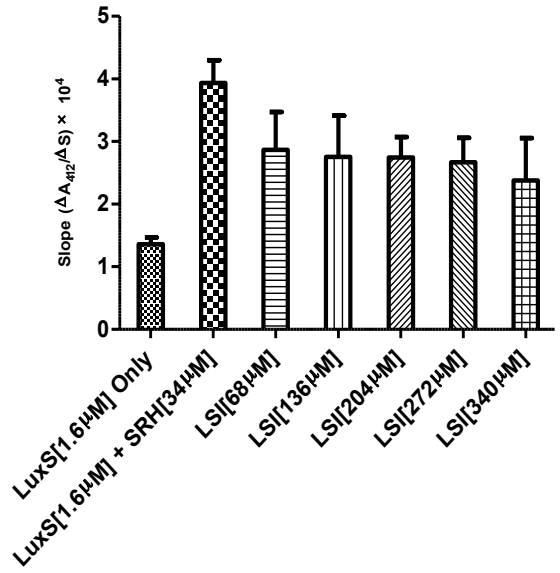


B

	LuxS [1.6 μM] Only	SRH [34 μM] Only	LuxS [1.6 μM] + SRH [34 μM]
Slope	$1.4 (\pm 0.24) \times 10^{-4}$	$1.3 (\pm 0.31) \times 10^{-5}$	$3.9 (\pm 0.26) \times 10^{-4}$

	LSI [68 μM]	LSI [136 μM]	LSI [204 μM]	LSI [272 μM]	LSI [340 μM]
Slope	$2.9 (\pm 0.23) \times 10^{-4}$	$2.8 (\pm 0.38) \times 10^{-4}$	$2.7 (\pm 0.25) \times 10^{-4}$	$2.7 (\pm 0.28) \times 10^{-4}$	$2.4 (\pm 0.23) \times 10^{-4}$

C



	LuxS [1.6 μM] Only	LuxS [1.6 μM] + SRH [34 μM]	LSI [68 μM]	LSI [136 μM]	LSI [204 μM]	LSI [272 μM]	LSI [340 μM]
Standard Error	0.11	0.36	0.61	0.66	0.33	0.39	0.68

Figure 6.3.4 | Sulfasalazine Inhibition Assay. Dose-dependent inhibition trials for sulfasalazine. (A) Average of 3 LuxS inhibition assay runs using sulfasalazine as the possible inhibitor. The error bars given on the graph are in standard error of 3 trials. (Legend: LSI = LuxS + SRH + Inhibitor , closed circle = Buffer Blank, closed square = LuxS[1.6 μM] only, closed triangle = SRH[34 μM] only, closed inverted triangle = LuxS[1.6 μM] + SRH[34 μM], closed diamond = LSI[68 μM], open circle = LSI[136 μM], open square = [204 μM], open triangle = LSI[272 μM], and open inverted triangle = LSI[340 μM]) (B) Shows the best-fit line for each subject using sulfasalazine as the inhibitor. C) Slopes from each LuxS inhibition run used to compare the activity under different concentrations of sulfasalazine. The column is produced from the average slope of each subject from all 3 trials. Error bars that are on the graph are the standard error of 3 trials for each subject.

6.4 | Determination of IC_{50}. The IC_{50} values were determined for substrates that showed promise in inhibition of the LuxS enzyme from the Ellman's assay. It was imperative that the IC_{50} was determined so that it can be seen how much of the potential inhibitor is needed to inhibit the LuxS enzyme by fifty percent. The IC_{50} of argininosuccinate and L-NAME were calculated and determined. Equation for the calculation of the IC_{50} is shown below.

$$\%\ Inhibition\ = \left[\frac{(normal\ activity\ -\ inhibited\ activity)}{(normal\ activity)}\right] \times 100\%$$

$$100\ -\ \%\ inhibition\ =\ \%\ Activity$$

6.4.1 | Argininosuccinate. Argininosuccinate was the first substrate to have an IC_{50} graph be completed. However, the graph is not a true triplicate due to the lack of reagent when it was initially performed. The average of three different runs of the Argininosuccinate was used to calculate the IC_{50} average.

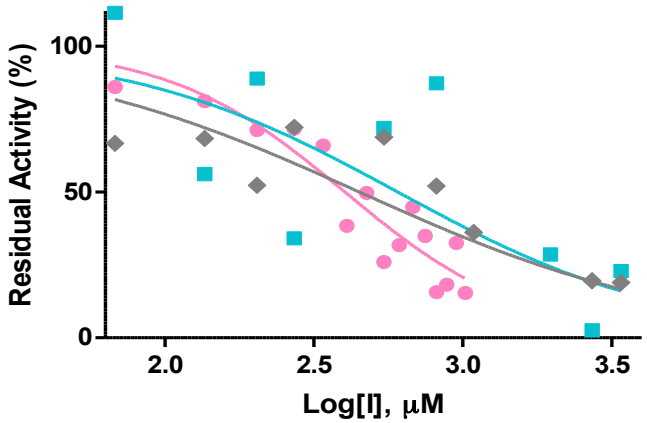
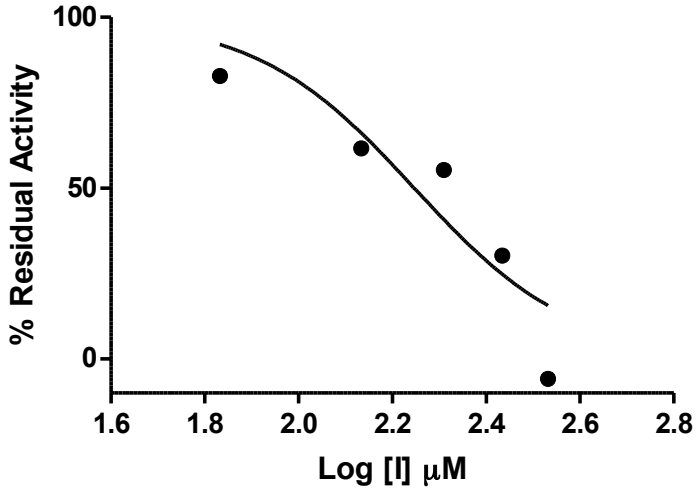


Figure 6.4.1 | Argininosuccinate IC_{50} Determination. The average IC_{50} of argininosuccinate from the three individual run was 490 μM. Individually, the IC_{50} of the first trial (Pink) is 400.0 with a $LogIC_{50}$ standard error of 0.036. The second trial (Turquoise) has an IC_{50} of 610 with a $LogIC_{50}$ standard error of 0.21 and the third trial (Grey) has an IC_{50} of 450 with a $LogIC_{50}$ standard error of 0.16.

6.4.2 | L-Nitroarginine Methyl Ester Hydrochloride (L-NAME). The most promising potential inhibitor that was ever tested to date is L-NAME. Compared to the Argininosuccinate, the L-NAME is twice more potent as an inhibitor of LuxS according to the Ellman's assay. It has an IC_{50} average of 180 μM.



IC_{50} [μM]	$LogIC_{50}$ [μM]
180	2.2

Figure 6.4.2 | L-NAME IC_{50} Determination. The IC_{50} graph of L-NAME. The curve is produced from the average slope of each point from all (3) trials. Error bars that are on the graph are the standard error of (3) trials for each subject.

6.5 | Conclusion and Future Directions. To date, there have been no other research groups known to search for LuxS inhibitors through computational screening. What is further unique to this project is that the screening was done through an automated computational screening program called DOCK Blaster, which contains different databases to screen for inhibitors. Using this methodology, it allowed for accessibility for those who do not have a broad background in computational chemistry. The screening gave results that showed potential competitive inhibition. The most promising inhibitor from the screening result to this date is compound L-nitroarginine methyl ester hydrochloride (L-NAME), which showed an IC_{50} of 180 μM (Figure 6.4.2) compared to its initial predecessor, argininosuccinate, which has an average IC_{50} of 490 μM. From the results given by DOCK Blaster, it has been demonstrated that searching for potential competitive inhibitors through computational screening is viable. Currently, further testing of other arginine based structures are screened through the inhibition assay to determine whether other arginine structures show inhibition of quorum sensing system two as well. Furthermore, future work can be done by continuously screening for competitive inhibitors using other databases that are within DOCK Blaster and for those who have a background in computational screening, inhibitors can be designed manually to fit the LuxS enzyme active site.

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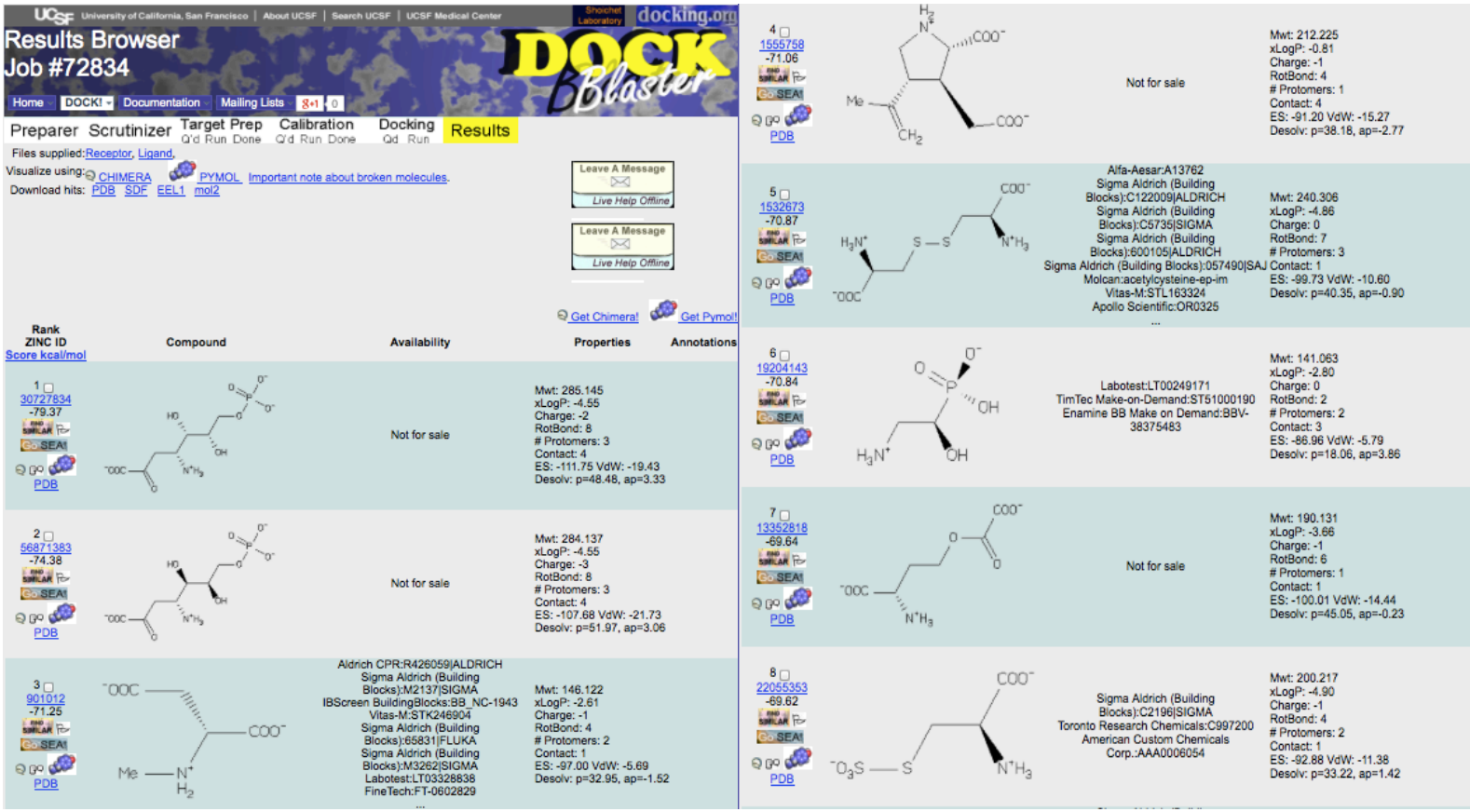
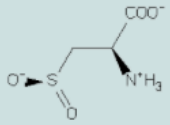
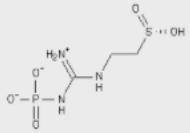
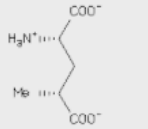
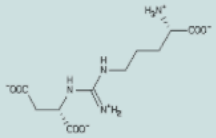
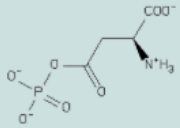
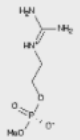
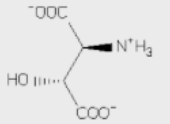
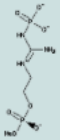
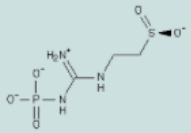
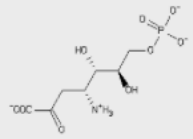
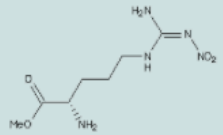
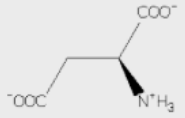
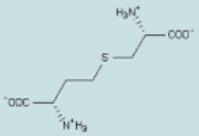
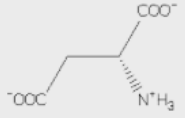
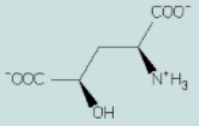
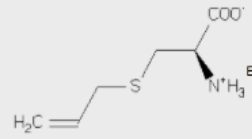
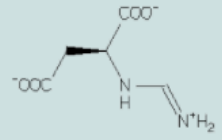
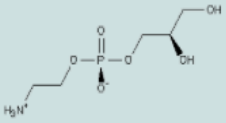
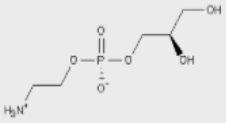
Appendix A: DOCK Blaster Screening Runs

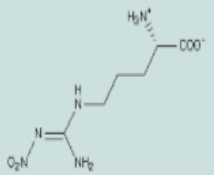
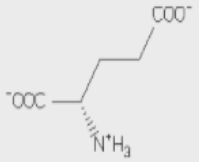
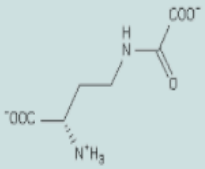
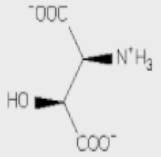
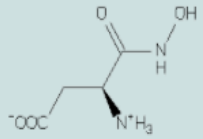
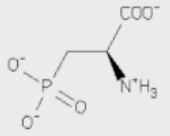
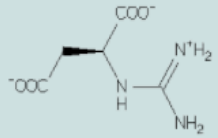
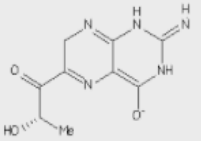
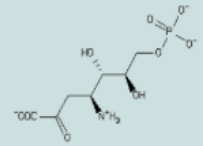
Results from the docking of 1JVI PDB code: 72834. The re-docking of the SRH onto the LuxS active site showed great results (in green). This run was done using the faster scheme run vs. the slower scheme. Below is the result page that contains 200 potential inhibitors for the LuxS enzyme according the KEGG database. SRH re-docked as a result from the KEGG database, ranking at 126. Potentially, this means that there are 125 other substrates that could potentially bind to the LuxS active site more competitively than the SRH substrate.

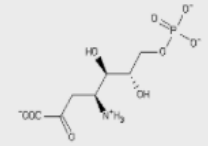
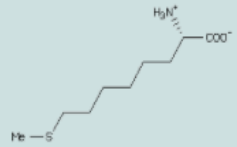
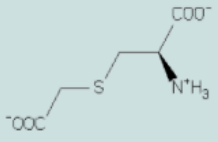
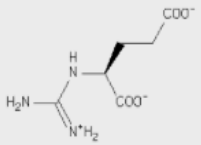
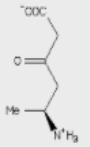
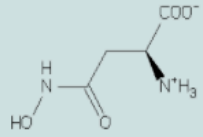
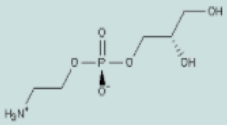
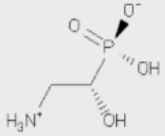
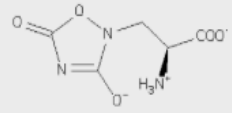
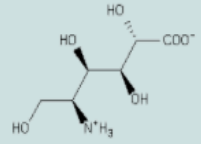
		Scoring	
		Polarized	AMBER
Sampling	Coarser	1.491 / 0	2.502 / 7
	Finer	1.265 / 0	1.51 / 6

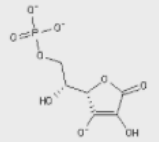
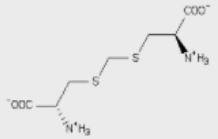
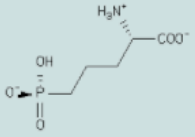
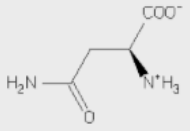
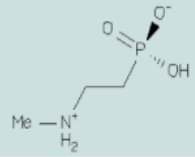
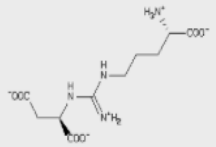
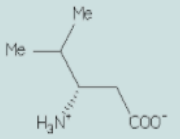
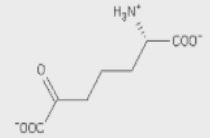
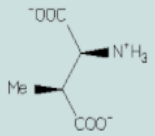

UCSF University of California, San Francisco | About UCSF | Search UCSF | UCSF Medical Center
Shoichet Laboratory
docking.org
Results Browser
Job #72834
DOCK Blaster
Home
DOCK!
Documentation
Mailing Lists
Preparer
Scrutinizer
Target Prep
Q'd Run Done
Calibration
Q'd Run Done
Docking
Qd Run
Results
Files supplied: Receptor, Ligand,
Visualize using: CHIMERA PYMOL Important note about broken molecules.
Download hits: PDB SDF EEL1 mol2
Leave A Message
Live Help Offline
Leave A Message
Live Help Offline
Get Chimera!
Get Pymol!
Rank
ZINC ID
Score kcal/mol
Compound
Availability
Properties
Annotations
1
30727834
-79.37
PDB
Not for sale
Mwt: 285.145
xLogP: -4.55
Charge: -2
RotBond: 8
Protomers: 3
Contact: 4
ES: -111.75 VdW: -19.43
Desolv: p=48.48, ap=3.33
2
56871383
-74.38
PDB
Not for sale
Mwt: 284.137
xLogP: -4.55
Charge: -3
RotBond: 8
Protomers: 3
Contact: 4
ES: -107.68 VdW: -21.73
Desolv: p=51.97, ap=3.06
3
901012
-71.25
PDB
Aldrich CPR:R426059|ALDRICH
Sigma Aldrich (Building Blocks):M2137|SIGMA
IBScreen BuildingBlocks:BB_NC-1943
Vitas-M:STK246904
Sigma Aldrich (Building Blocks):65831|FLUKA
Sigma Aldrich (Building Blocks):M3262|SIGMA
Labotest:LT03328838
FineTech:FT-0602829
Mwt: 146.122
xLogP: -2.61
Charge: -1
RotBond: 4
Protomers: 2
Contact: 1
ES: -97.00 VdW: -5.69
Desolv: p=32.95, ap=-1.52
4
1555758
-71.06
PDB
Not for sale
Mwt: 212.225
xLogP: -0.81
Charge: -1
RotBond: 4
Protomers: 1
Contact: 4
ES: -91.20 VdW: -15.27
Desolv: p=38.18, ap=-2.77
5
1532673
-70.87
PDB
Alfa-Aesar:A13762
Sigma Aldrich (Building Blocks):C122009|ALDRICH
Sigma Aldrich (Building Blocks):C5735|SIGMA
Sigma Aldrich (Building Blocks):600105|ALDRICH
Sigma Aldrich (Building Blocks):057490|SAJ
Molcan:acetylcysteine-ep-im
Vitas-M:STL163324
Apollo Scientific:OR0325
...
Mwt: 240.306
xLogP: -4.86
Charge: 0
RotBond: 7
Protomers: 3
Contact: 1
ES: -99.73 VdW: -10.60
Desolv: p=40.35, ap=-0.90
6
19204143
-70.84
PDB
Labotest:LT00249171
TimTec Make-on-Demand:ST51000190
Enamine BB Make on Demand:BBV-38375483
Mwt: 141.063
xLogP: -2.80
Charge: 0
RotBond: 2
Protomers: 2
Contact: 3
ES: -86.96 VdW: -5.79
Desolv: p=18.06, ap=3.86
7
13352818
-69.64
PDB
Not for sale
Mwt: 190.131
xLogP: -3.66
Charge: -1
RotBond: 6
Protomers: 1
Contact: 1
ES: -100.01 VdW: -14.44
Desolv: p=45.05, ap=-0.23
8
22055353
-69.62
PDB
Sigma Aldrich (Building Blocks):C2196|SIGMA
Toronto Research Chemicals:C997200
American Custom Chemicals Corp.:AAA0006054
Mwt: 200.217
xLogP: -4.90
Charge: -1
RotBond: 4
Protomers: 2
Contact: 1
ES: -92.88 VdW: -11.38
Desolv: p=33.22, ap=1.42

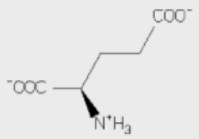
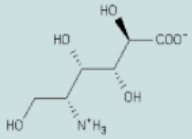
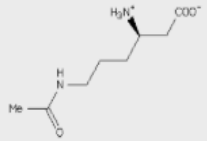
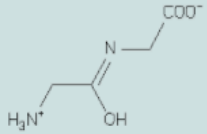
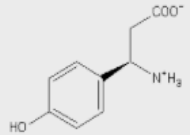
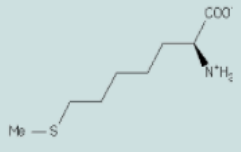
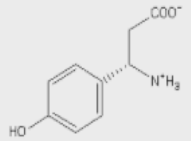
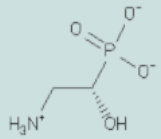
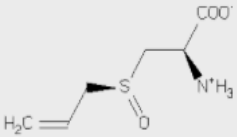
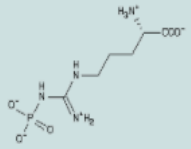
9 3869282 -69.03 PDB		Sigma Aldrich (Building Blocks):C4418|SIGMA Princeton BioMolecular Research:OSSL_298271 Pharmeks:PHAR040154 Sigma Aldrich (Building Blocks):270881|ALDRICH Labotest:LT00452943 Ambinter Natural Products:GPL000369 eMolecules:536121 TimTec Make-on-Demand:ST51006991 ...	Mwt: 152.151 xLogP: -4.87 Charge: -1 RotBond: 3 # Protomers: 1 Contact: 1 ES: -97.60 VdW: -9.48 Desolv: p=36.73, ap=1.32	14 15265482 -66.03 PDB		Not for sale	Mwt: 230.162 xLogP: -4.08 Charge: -1 RotBond: 6 # Protomers: 2 Contact: 3 ES: -76.63 VdW: -19.55 Desolv: p=27.21, ap=2.94
10 896091 -67.82 PDB		Toronto Research Chemicals:M311575 Sigma Aldrich (Building Blocks):G137|SIGMA FineTech:FT-0604674 Princeton BioMolecular BuildingBlocks:PBMR052902 American Custom Chemicals Corp.:AAA0000080	Mwt: 160.149 xLogP: -2.77 Charge: -1 RotBond: 4 # Protomers: 1 Contact: 1 ES: -100.76 VdW: -6.97 Desolv: p=41.12, ap=-1.21	15 1529646 -65.63 PDB		Sigma Aldrich (Building Blocks):A5707|ALDRICH	Mwt: 289.268 xLogP: -4.67 Charge: -1 RotBond: 11 # Protomers: 1 Contact: 1 ES: -119.01 VdW: -18.02 Desolv: p=67.40, ap=4.01
11 3870028 -67.57 PDB		Not for sale	Mwt: 211.066 xLogP: -2.31 Charge: -2 RotBond: 5 # Protomers: 1 Contact: 1 ES: -140.20 VdW: -10.59 Desolv: p=81.76, ap=1.47	16 6483376 -65.53 PDB		Not for sale	Mwt: 197.131 xLogP: -1.98 Charge: 0 RotBond: 5 # Protomers: 1 Contact: 1 ES: -83.62 VdW: -10.91 Desolv: p=29.17, ap=-0.17
12 901877 -67.09 PDB		Princeton BioMolecular Research:OSSL_298282 eMolecules:749478 Ambinter Natural Products:Ambmdy00501000 Anward:ANW-36077 Molport:MolPort-003-665-668 Scientific Exchange (make on demand):F-393884 Tractus:RT-001415 TCI:H0947 ...	Mwt: 147.086 xLogP: -4.36 Charge: -2 RotBond: 3 # Protomers: 2 Contact: 2 ES: -91.26 VdW: -9.56 Desolv: p=32.88, ap=0.85	17 31970429 -64.74 PDB		Not for sale	Mwt: 276.102 xLogP: -2.76 Charge: -1 RotBond: 8 # Protomers: 4 Contact: 2 ES: -87.57 VdW: -16.06 Desolv: p=38.34, ap=0.55
13 15265484 -66.94 PDB		Not for sale	Mwt: 230.162 xLogP: -4.20 Charge: -1 RotBond: 5 # Protomers: 6 Contact: 3 ES: -77.09 VdW: -18.13 Desolv: p=25.53, ap=2.75	18 56871381 -64.67 PDB		Not for sale	Mwt: 284.137 xLogP: -4.55 Charge: -3 RotBond: 8 # Protomers: 3 Contact: 4 ES: -98.60 VdW: -21.42 Desolv: p=51.75, ap=3.59

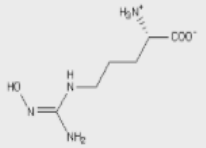
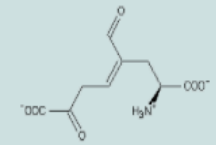
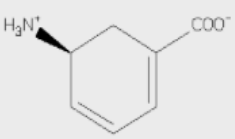
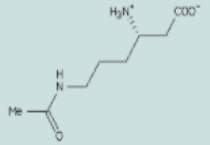
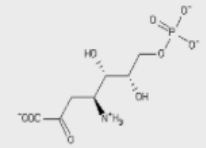
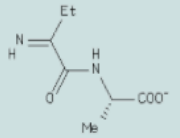
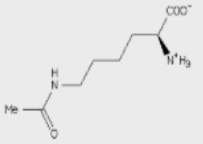
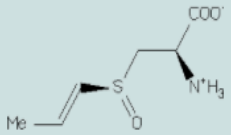
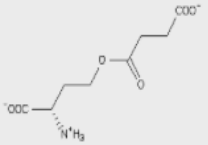
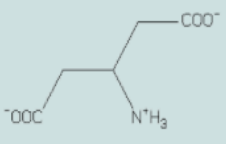
19 15987659 -64.40 PDB		TimTec Building Blocks:SBB001059 Scientific Exchange (make on demand):K-014774 Sigma Aldrich (Building Blocks):N4770|SIGMA Princeton BioMolecular Research:OSSL_298356 Molport BB:MolPort-000-655-958 Molport:MolPort-019-736-186 Sigma Aldrich (Building Blocks):72760|FLUKA Sigma Aldrich (Building Blocks):N5751|SIGMA ...	Mwt: 232.22 xLogP: -1.56 Charge: -1 RotBond: 8 # Protomers: 7 Contact: 1 ES: -81.82 VdW: -18.17 Desolv: p=36.08, ap=-0.49
20 895032 -63.70 PDB		Sigma Aldrich (Building Blocks):332135|ALDRICH NCI Plated 2007:97922 NCI Plated 2007:79553 Sigma Aldrich (Building Blocks):A5474|SIGMA Alfa-Aesar:43317 Sigma Aldrich (Building Blocks):51572|FLUKA Sigma Aldrich (Building Blocks):589667|ALDRICH Sigma Aldrich (Building Blocks):11250|ALDRICH ...	Mwt: 133.103 xLogP: -3.52 Charge: 0 RotBond: 3 # Protomers: 2 Contact: 1 ES: -87.26 VdW: -9.59 Desolv: p=33.64, ap=-0.48
21 1532680 -63.26 PDB		Sigma Aldrich (Building Blocks):15984|FLUKA Princeton BioMolecular Research:OSSL_297345 Scientific Exchange (make on demand):F-137764 Aldrich CPR:S788988|ALDRICH Ambinter Natural Products:GPL000152 FineTech:FT-0627595 TimTec:ST086627 eMolecules:536031 ...	Mwt: 222.266 xLogP: -4.46 Charge: 0 RotBond: 7 # Protomers: 1 Contact: 1 ES: -104.29 VdW: -14.72 Desolv: p=51.91, ap=3.85
22 895218 -63.25 PDB		Alfa-Aesar:A13646 Sigma Aldrich (Building Blocks):488941|ALDRICH Sigma Aldrich (Building Blocks):589667|ALDRICH Sigma Aldrich (Building Blocks):11240|ALDRICH Alfa-Aesar:B21184 Labotest:LT03328272 IBScreen BuildingBlocks:BB_NC-2214 TimTec Building Blocks:SBB015070 ...	Mwt: 133.103 xLogP: -3.52 Charge: 0 RotBond: 3 # Protomers: 2 Contact: 1 ES: -91.42 VdW: -4.35 Desolv: p=32.95, ap=-0.43
23 895967 -63.19 PDB		Sigma Aldrich (Building Blocks):76157|SIGMA Toronto Research Chemicals:H942550 Ambinter Natural Products:GPL000209 Bachem:F-3335 American Custom Chemicals Corp.:CCH0007792	Mwt: 162.121 xLogP: -2.19 Charge: -1 RotBond: 4 # Protomers: 1 Contact: 2 ES: -103.92 VdW: -6.59 Desolv: p=44.56, ap=2.76
24 2517162 -63.09 PDB		Innovapharm BB Make on Demand:BBV-00038867 Sigma Aldrich (Building Blocks):616052|ALDRICH UORSY BB Make-on-demand:BBV-32656751 Enamine BB Make on Demand:BBV-076234 ChiralBlock BioScience BB:485 Sigma Aldrich (Building Blocks):SML0337|SIGMA Anward:ANW-24504 Anward:ANW-73156 ...	Mwt: 161.226 xLogP: -1.86 Charge: 0 RotBond: 5 # Protomers: 2 Contact: 1 ES: -69.94 VdW: -12.82 Desolv: p=21.24, ap=-1.58
25 4096096 -63.03 PDB		Not for sale	Mwt: 159.121 xLogP: -3.00 Charge: -1 RotBond: 5 # Protomers: 1 Contact: 1 ES: -95.30 VdW: -12.36 Desolv: p=43.71, ap=0.92
26 56871085 -62.81 PDB		Not for sale	Mwt: 228.161 xLogP: -2.57 Charge: -1 RotBond: 8 # Protomers: 2 Contact: 2 ES: -97.21 VdW: -6.76 Desolv: p=38.98, ap=2.18
27 4095590 -62.75 PDB		Ambinter Natural Products:GPL000093 Anward:ANW-60230 BePharm Building Blocks:B232924 Ark Pharm Building Blocks:AK101385 Molport BB:MolPort-006-169-092	Mwt: 215.142 xLogP: -3.18 Charge: 0 RotBond: 7 # Protomers: 1 Contact: 3 ES: -99.87 VdW: -6.53 Desolv: p=39.00, ap=4.64
28 12503696 -62.49 PDB		Ambinter Natural Products:GPL000093 Anward:ANW-60230 BePharm Building Blocks:B232924 Ark Pharm Building Blocks:AK101385 Molport BB:MolPort-006-169-092	Mwt: 215.142 xLogP: -3.18 Charge: 0 RotBond: 7 # Protomers: 1 Contact: 3 ES: -96.86 VdW: -8.96 Desolv: p=39.86, ap=3.47

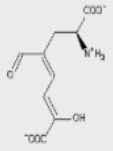
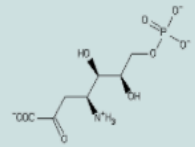
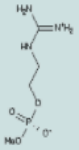
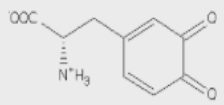
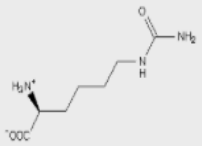
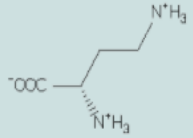
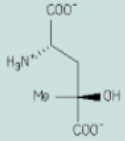
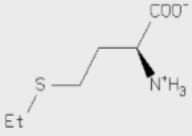
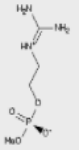
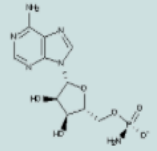
29 19796052 -62.48 PDB		Sigma Aldrich (Building Blocks):72750|FLUKA FineTech:FT-0629730 eMolecules:529712 TimTec Make-on-Demand:ST50975058 Indofine:04-2167 Cayman Chemical:80220 Princeton BioMolecular Research:OSSL_108087 ChemDiv BuildingBlocks:BB55-8569 ...	Mwt: 218.193 xLogP: -3.63 Charge: -1 RotBond: 7 # Protomers: 4 Contact: 1 ES: -80.45 VdW: -18.77 Desolv: p=37.35, ap=-0.61
30 1482113 -62.33 PDB		Sigma Aldrich (Building Blocks):486574|ALDRICH NCI Plated 2007:88574 NCI Plated 2007:77686 Sigma Aldrich (Building Blocks):G5667|SIGMA ChemBridge:5102620 Sigma Aldrich (Building Blocks):749435|ALDRICH Sigma Aldrich (Building Blocks):607851|ALDRICH Sigma Aldrich (Building Blocks):49605|ALDRICH ...	Mwt: 146.122 xLogP: -3.25 Charge: -1 RotBond: 4 # Protomers: 1 Contact: 1 ES: -97.35 VdW: -8.54 Desolv: p=44.38, ap=-0.83
31 1531039 -62.32 PDB		Not for sale	Mwt: 189.147 xLogP: -3.11 Charge: -1 RotBond: 5 # Protomers: 1 Contact: 1 ES: -98.40 VdW: -14.70 Desolv: p=47.78, ap=3.00
32 901878 -62.09 PDB		Princeton BioMolecular Research:OSSL_298282 Toronto Research Chemicals:H828525 Scientific Exchange (make on demand):F-393884 FineTech:FT-0625518 eMolecules:749478 Ambinter Natural Products:Ambmdy00501000 Anward:ANW-36077 Molport:MolPort-003-665-668 ...	Mwt: 148.094 xLogP: -4.36 Charge: -1 RotBond: 3 # Protomers: 2 Contact: 2 ES: -87.04 VdW: -10.01 Desolv: p=34.38, ap=0.57
33 4096026 -61.92 PDB		UORSY BB Make-on-demand:BBV-39239950 Enamine BB Make on Demand:BBV-39239950	Mwt: 148.118 xLogP: -2.52 Charge: 0 RotBond: 3 # Protomers: 2 Contact: 2 ES: -79.32 VdW: -12.26 Desolv: p=26.74, ap=2.92
34 3873028 -61.87 PDB		Apollo Scientific:OR10671 NCI Plated 2007:30078 IBScreen Bioactives:Bio-0618	Mwt: 168.065 xLogP: -4.26 Charge: -1 RotBond: 3 # Protomers: 2 Contact: 2 ES: -86.59 VdW: -8.56 Desolv: p=31.90, ap=1.38
35 4096031 -61.75 PDB		Sigma Aldrich (Building Blocks):G7379|SIGMA	Mwt: 174.136 xLogP: -3.53 Charge: -1 RotBond: 5 # Protomers: 1 Contact: 1 ES: -94.88 VdW: -12.47 Desolv: p=43.31, ap=2.29
36 17721961 -61.65 PDB		Sigma Aldrich (Building Blocks):S154|ALDRICH TimTec Make-on-Demand:ST50405213 Molport BB:MolPort-009-018-798	Mwt: 237.219 xLogP: -1.25 Charge: 0 RotBond: 2 # Protomers: 9 Contact: 2 ES: -50.01 VdW: -22.60 Desolv: p=10.70, ap=0.26
37 56871380 -61.61 PDB		Not for sale	Mwt: 284.137 xLogP: -4.55 Charge: -3 RotBond: 8 # Protomers: 3 Contact: 4 ES: -105.16 VdW: -18.12 Desolv: p=58.00, ap=3.67

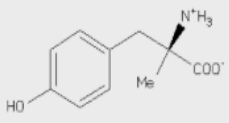
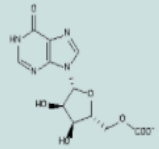
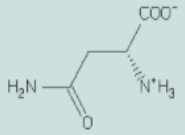
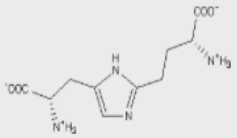
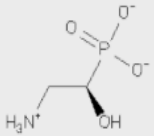
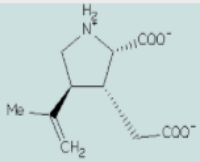
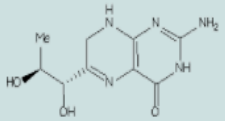
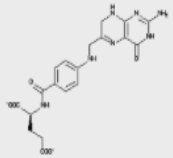
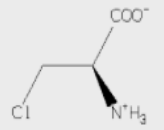
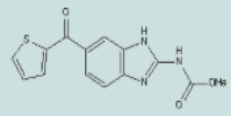
38 30727820 -61.43 PDB		Not for sale	Mwt: 285.145 xLogP: -4.55 Charge: -2 RotBond: 8 # Protomers: 3 Contact: 4 ES: -98.92 VdW: -18.56 Desolv: p=51.74, ap=4.30	43 56874420 -61.00 PDB		Not for sale	Mwt: 204.315 xLogP: -0.45 Charge: -1 RotBond: 8 # Protomers: 2 Contact: 1 ES: -72.15 VdW: -12.96 Desolv: p=27.17, ap=-3.04
39 1529732 -61.20 PDB		Alfa-Aesar:B23487 Sigma Aldrich (Building Blocks):851213|ALDRICH TimTec Building Blocks:SBB015059 BioSynth:C-1850 Enamine BB Make on Demand:BBV-38737533 Sigma Aldrich (Building Blocks):C7757|SIGMA Labotest:LT00244766 FineTech:FT-0602920 ...	Mwt: 178.189 xLogP: -3.26 Charge: -1 RotBond: 5 # Protomers: 1 Contact: 1 ES: -93.96 VdW: -10.84 Desolv: p=41.47, ap=2.13	44 1529598 -60.95 PDB		Sigma Aldrich (Building Blocks):G7788|SIGMA Cayman Chemical:80340 Anward:ANW-36349 Tractus:TR-023852 American Custom Chemicals Corp.:NUC0003022 TCI:G0265 Molport BB:MolPort-009-018-784 ...	Mwt: 188.163 xLogP: -3.26 Charge: -1 RotBond: 6 # Protomers: 2 Contact: 1 ES: -97.52 VdW: -6.79 Desolv: p=44.95, ap=-1.58
40 901794 -61.15 PDB			Mwt: 145.158 xLogP: -1.52 Charge: 0 RotBond: 4 # Protomers: 1 Contact: 1 ES: -89.89 VdW: -9.57 Desolv: p=37.22, ap=1.09	45 25695750 -60.94 PDB		Sigma Aldrich (Building Blocks):A6508|SIGMA American Custom Chemicals Corp.:AAA0006157	Mwt: 148.118 xLogP: -2.75 Charge: 0 RotBond: 3 # Protomers: 2 Contact: 2 ES: -82.67 VdW: -9.21 Desolv: p=28.61, ap=2.34
41 4095591 -61.10 PDB		Anward:ANW-60230 BePharm Building Blocks:B232924 Ark Pharm Building Blocks:AK101385 Molport BB:MolPort-006-169-092	Mwt: 215.142 xLogP: -3.18 Charge: 0 RotBond: 7 # Protomers: 1 Contact: 3 ES: -100.37 VdW: -5.33 Desolv: p=39.55, ap=5.05	46 19204141 -60.88 PDB		Labotest:LT00249171 TimTec Make-on-Demand:ST51000190 Enamine BB Make on Demand:BBV-38375483	Mwt: 141.063 xLogP: -2.80 Charge: 0 RotBond: 2 # Protomers: 2 Contact: 3 ES: -85.11 VdW: -6.04 Desolv: p=26.55, ap=3.72
42 13779018 -61.07 PDB		Sigma Aldrich (Building Blocks):Q2128|SIGMA Ambinter Natural Products:GPN000665 FineTech:FT-0604435 Fluorochem:M02436 ChemMol:97900382 TimTec Make-on-Demand:ST50825297 AK Scientific:V0329 Molport BB:MolPort-003-939-212 ...	Mwt: 187.111 xLogP: -4.04 Charge: -2 RotBond: 3 # Protomers: 3 Contact: 2 ES: -77.07 VdW: -10.31 Desolv: p=23.47, ap=2.83	47 4096196 -60.84 PDB		Not for sale	Mwt: 195.171 xLogP: -3.93 Charge: 0 RotBond: 5 # Protomers: 1 Contact: 5 ES: -89.04 VdW: -13.26 Desolv: p=35.59, ap=5.86

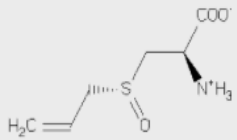
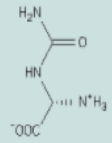
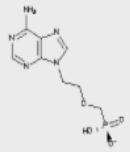
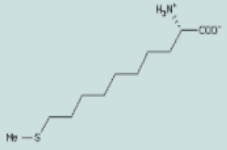
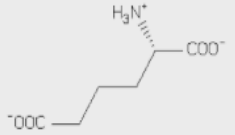
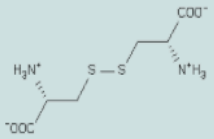
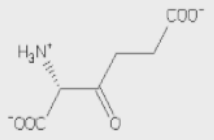
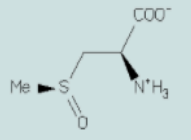
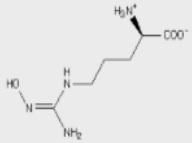
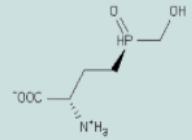
48 30730324 -60.83 PDB		Not for sale	Mwt: 254.087 xLogP: -4.59 Charge: 0 RotBond: 4 # Protomers: 5 Contact: 2 ES: -81.74 VdW: -18.04 Desolv: p=36.17, ap=2.77
49 56874438 -60.83 PDB		Not for sale	Mwt: 218.342 xLogP: 0.05 Charge: -1 RotBond: 9 # Protomers: 2 Contact: 1 ES: -70.58 VdW: -12.93 Desolv: p=26.19, ap=-3.52
50 1531043 -60.50 PDB		NCI Plated 2007:76076 Aldrich CPR:S449229|ALDRICH Ambinter Natural Products:Ambmdy00310008 Sequoia Research Products:498-59-9 Apollo Scientific Bioactives:BID1201 American Custom Chemicals Corp.:API0003139 TCI:D0961 ...	Mwt: 254.333 xLogP: -4.85 Charge: 0 RotBond: 8 # Protomers: 3 Contact: 1 ES: -83.95 VdW: -16.25 Desolv: p=40.61, ap=-0.92
51 19632959 -60.41 PDB		IBScreen Bioactives:Bio-0871 Sigma Aldrich (Building Blocks):283967|ALDRICH Sigma Aldrich (Building Blocks):A6553|SIGMA Sigma Aldrich (Building Blocks):09248|FLUKA ZereneX Building Blocks:ZBioX-0712 Fluorochem:M01268 Pharmeks:PHAR177799 Pharmeks:PHAR031188 ...	Mwt: 196.119 xLogP: -3.75 Charge: -1 RotBond: 5 # Protomers: 1 Contact: 2 ES: -92.38 VdW: -7.86 Desolv: p=39.19, ap=0.64
52 1532556 -60.32 PDB		Maybridge Building Blocks:SB01192 Sigma Aldrich (Building Blocks):570745|ALDRICH Sigma Aldrich (Building Blocks):750824|ALDRICH Sigma Aldrich (Building Blocks):608157|ALDRICH Sigma Aldrich (Building Blocks):11150|FLUKA Apollo Scientific:OR28751 Sigma Aldrich (Building Blocks):A4159|SIGMA Alfa-Aesar:A15012 ...	Mwt: 132.119 xLogP: -2.81 Charge: 0 RotBond: 3 # Protomers: 1 Contact: 1 ES: -83.08 VdW: -3.99 Desolv: p=23.67, ap=3.09
53 56871180 -60.18 PDB			Mwt: 139.091 xLogP: -1.16 Charge: 0 RotBond: 3 # Protomers: 1 Contact: 2 ES: -80.84 VdW: -4.36 Desolv: p=24.03, ap=0.98
54 1529647 -59.69 PDB		Not for sale	Mwt: 289.268 xLogP: -4.67 Charge: -1 RotBond: 11 # Protomers: 1 Contact: 1 ES: -116.57 VdW: -15.14 Desolv: p=67.85, ap=4.16
55 170090 -59.62 PDB		ChemMol:44019441 Key Organics Building Blocks:7T-0026 Sigma Aldrich (Building Blocks):17988|ALDRICH Fluorochem:040070 FineTech:FT-0630332 eMolecules:485964 Matrix Scientific:038631 Oakwood Chemical:040070 ...	Mwt: 131.175 xLogP: -0.19 Charge: 0 RotBond: 3 # Protomers: 1 Contact: 1 ES: -77.40 VdW: -9.05 Desolv: p=27.47, ap=-0.64
56 1529771 -59.39 PDB		Not for sale	Mwt: 188.159 xLogP: -3.57 Charge: -1 RotBond: 6 # Protomers: 1 Contact: 1 ES: -95.76 VdW: -13.84 Desolv: p=48.68, ap=1.53
57 901781 -59.25 PDB		NCI Plated 2007:45365 Sigma Aldrich (Building Blocks):M6126|SIGMA Princeton BioMolecular Research:OSSL_297264 Scientific Exchange (make on demand):F-137682 eMolecules:592092 Labotest:LT00772127 Molport:MolPort-003-958-793 Capot Chemical:10166 ...	Mwt: 146.122 xLogP: -3.04 Charge: -1 RotBond: 3 # Protomers: 1 Contact: 1 ES: -98.95 VdW: 2.58 Desolv: p=35.56, ap=1.56

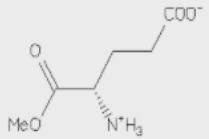
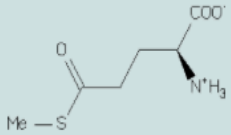
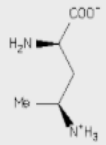
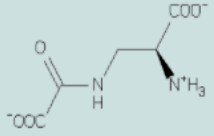
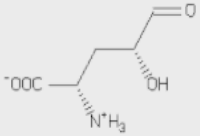
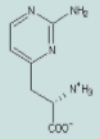
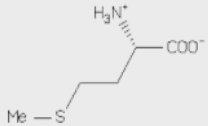
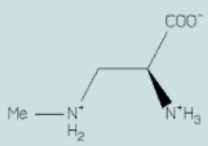
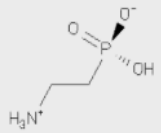
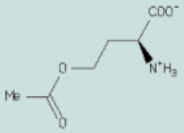
58 895124 -59.24 PDB		Sigma Aldrich (Building Blocks):486574|ALDRICH Sigma Aldrich (Building Blocks):749435|ALDRICH Sigma Aldrich (Building Blocks):587664|ALDRICH Sigma Aldrich (Building Blocks):49480|FLUKA Vitas-M:STK017280 Alfa-Aesar:A14191 Alfa-Aesar:A17719 Labotest:LT03328670 ...	Mwt: 146.122 xLogP: -3.25 Charge: -1 RotBond: 4 # Protomers: 1 Contact: 1 ES: -101.98 VdW: -2.13 Desolv: p=45.70, ap=-0.83
59 8551625 -59.20 PDB		Not for sale	Mwt: 195.171 xLogP: -3.93 Charge: 0 RotBond: 5 # Protomers: 1 Contact: 5 ES: -84.43 VdW: -15.95 Desolv: p=35.30, ap=5.87
60 1529764 -58.66 PDB		Not for sale	Mwt: 188.227 xLogP: -1.62 Charge: 0 RotBond: 6 # Protomers: 1 Contact: 1 ES: -80.01 VdW: -16.15 Desolv: p=34.82, ap=2.68
61 5112515 -58.65 PDB		NCI Plated 2007:49346 Alfa-Aesar:A10523 BioSynth:G-6000 Labotest:LT00005695	Mwt: 132.119 xLogP: -3.26 Charge: 0 RotBond: 3 # Protomers: 1 Contact: 2 ES: -95.72 VdW: -5.60 Desolv: p=38.27, ap=4.41
62 170331 -58.64 PDB		Innovapharm BB Make on Demand:BBV-00022892 PepTech:AL1056-1 Innovapharm Make-on-Demand:VT-00001953 ChemMol:44019537 TimTec Building Blocks:SBB017166 Key Organics Building Blocks:8R-0644 Florida Heterocyclic Compounds:25973 eMolecules:975654 ...	Mwt: 181.191 xLogP: -1.54 Charge: 0 RotBond: 3 # Protomers: 1 Contact: 3 ES: -71.90 VdW: -18.04 Desolv: p=28.89, ap=2.41
63 56874404 -58.64 PDB		Not for sale	Mwt: 190.288 xLogP: -0.96 Charge: -1 RotBond: 7 # Protomers: 2 Contact: 1 ES: -66.43 VdW: -15.61 Desolv: p=25.85, ap=-2.46
64 170333 -58.63 PDB		TimTec Building Blocks:SBB017166 Innovapharm BB Make on Demand:BBV-00022892 PepTech:AD1057-1 Innovapharm Make-on-Demand:VT-00001953 Key Organics Building Blocks:8R-0644 Florida Heterocyclic Compounds:25973 eMolecules:975654 ChemMol:30114843 ...	Mwt: 181.191 xLogP: -1.54 Charge: 0 RotBond: 3 # Protomers: 1 Contact: 3 ES: -72.40 VdW: -18.83 Desolv: p=29.91, ap=2.69
65 4096868 -58.56 PDB		Labotest:LT00249171	Mwt: 140.055 xLogP: -2.80 Charge: -1 RotBond: 2 # Protomers: 1 Contact: 2 ES: -101.21 VdW: -6.55 Desolv: p=45.78, ap=3.42
66 1531038 -58.54 PDB		Sigma Aldrich (Building Blocks):72805|FLUKA Molport:MolPort-021-804-608 Sigma Aldrich (Building Blocks):74264|SIGMA Ambinter Natural Products:GPN001032 eMolecules:43030631 Ambinter Natural Products:Ambmdy01505190 Indofine:027253S Ambinter Natural Products:NP-009885 ...	Mwt: 177.225 xLogP: -3.39 Charge: 0 RotBond: 5 # Protomers: 1 Contact: 1 ES: -75.05 VdW: -11.03 Desolv: p=25.19, ap=2.35
67 1530092 -58.50 PDB		American Custom Chemicals Corp.:AAA0000659 Toronto Research Chemicals:P354000	Mwt: 253.175 xLogP: -3.38 Charge: -1 RotBond: 8 # Protomers: 1 Contact: 1 ES: -109.55 VdW: -14.84 Desolv: p=61.66, ap=4.23

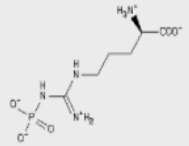
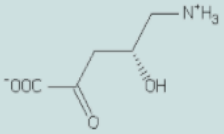
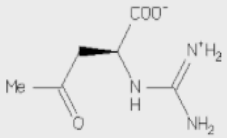
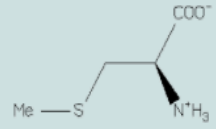
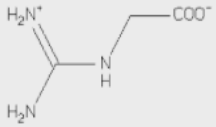
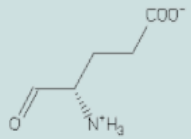
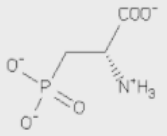
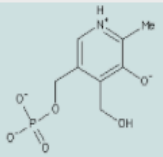
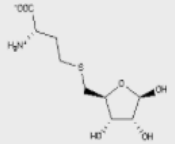
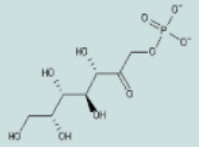
68 13558688 -58.38 PDB		Ambinter Natural Products:GPL000374 Toronto Research Chemicals:H943750	Mwt: 191.211 xLogP: -3.70 Charge: 1 RotBond: 6 # Protomers: 4 Contact: 2 ES: -68.50 VdW: -18.57 Desolv: p=27.98, ap=0.71
69 1530053 -58.31 PDB		Not for sale	Mwt: 228.18 xLogP: -3.80 Charge: -1 RotBond: 7 # Protomers: 1 Contact: 1 ES: -89.96 VdW: -14.42 Desolv: p=43.52, ap=2.55
70 968069 -58.23 PDB		Sigma Aldrich (Building Blocks):A3539|SIGMA Toronto Research Chemicals:G111000 Sigma Aldrich (Building Blocks):07710|FLUKA ChemMol:51504835 ChemMol:51504823	Mwt: 139.154 xLogP: -0.47 Charge: 0 RotBond: 1 # Protomers: 1 Contact: 2 ES: -86.88 VdW: -10.53 Desolv: p=40.24, ap=-1.05
71 1529763 -58.18 PDB		Not for sale	Mwt: 188.227 xLogP: -1.62 Charge: 0 RotBond: 6 # Protomers: 1 Contact: 1 ES: -73.69 VdW: -18.88 Desolv: p=31.91, ap=2.47
72 30727829 -58.17 PDB		Not for sale	Mwt: 285.145 xLogP: -4.55 Charge: -2 RotBond: 8 # Protomers: 3 Contact: 4 ES: -103.73 VdW: -17.19 Desolv: p=58.58, ap=4.16
73 56870898 -58.15 PDB		Not for sale	Mwt: 171.176 xLogP: -1.89 Charge: -1 RotBond: 4 # Protomers: 1 Contact: 2 ES: -64.26 VdW: -12.64 Desolv: p=19.50, ap=-0.74
74 1529433 -58.14 PDB		Maybridge:BTB13551 Aldrich CPR:S783579|ALDRICH TimTec Make-on-Demand:ST50307992 Labotest:LT00012645 Ambinter Natural Products:GPL000266 Indofine:04-2004 Sigma Aldrich (Building Blocks):A4021|SIGMA eMolecules:29549286 ...	Mwt: 188.227 xLogP: -2.84 Charge: 0 RotBond: 6 # Protomers: 1 Contact: 1 ES: -73.56 VdW: -18.64 Desolv: p=31.60, ap=2.47
75 1531055 -58.01 PDB			Mwt: 177.225 xLogP: -3.48 Charge: 0 RotBond: 4 # Protomers: 1 Contact: 1 ES: -78.45 VdW: -8.05 Desolv: p=25.99, ap=2.50
76 1532858 -57.92 PDB		Sigma Aldrich (Building Blocks):S7129|SIGMA ChiralBlock BioScience BB:376 American Custom Chemicals Corp.:AAA0003408 ChemMol:97600130 Apexmol Building Blocks:AM806589	Mwt: 218.185 xLogP: -3.42 Charge: -1 RotBond: 8 # Protomers: 1 Contact: 1 ES: -94.04 VdW: -15.47 Desolv: p=50.57, ap=1.02
77 895793 -57.88 PDB		PepTech:BL802-1 NCI Plated 2007:341646 Sigma Aldrich (Building Blocks):03688|FLUKA Sigma Aldrich (Building Blocks):G1763|SIGMA Fluorochem:045390 Chembo Pharma:KB-70424 Tractus:RT-011491 Zylexa Pharma BB:ZP-BB-PT000283 ...	Mwt: 146.122 xLogP: -1.79 Charge: -1 RotBond: 4 # Protomers: 1 Contact: 1 ES: -87.61 VdW: -8.46 Desolv: p=36.44, ap=1.75

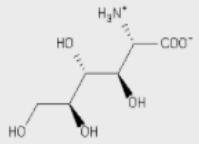
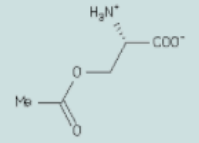
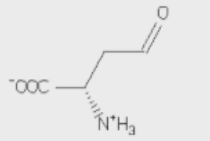
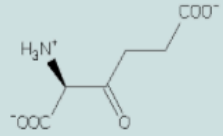
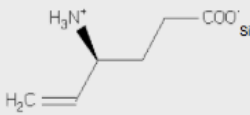
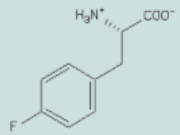
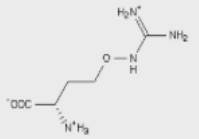
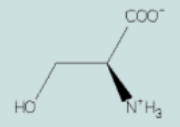
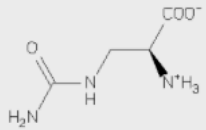
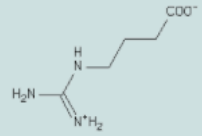
78 30725483 -57.86 PDB		Not for sale	Mwt: 227.172 xLogP: -3.06 Charge: -2 RotBond: 6 # Protomers: 3 Contact: 2 ES: -76.84 VdW: -12.51 Desolv: p=32.14, ap=-0.66	83 56871382 -57.66 PDB		Not for sale	Mwt: 284.137 xLogP: -4.55 Charge: -3 RotBond: 8 # Protomers: 3 Contact: 4 ES: -99.74 VdW: -13.88 Desolv: p=53.02, ap=2.95
79 13520273 -57.84 PDB		Not for sale	Mwt: 197.131 xLogP: -1.85 Charge: 0 RotBond: 6 # Protomers: 1 Contact: 1 ES: -73.70 VdW: -13.97 Desolv: p=29.85, ap=-0.01	84 1532756 -57.61 PDB			Mwt: 195.174 xLogP: -2.68 Charge: 0 RotBond: 3 # Protomers: 1 Contact: 2 ES: -73.69 VdW: -15.44 Desolv: p=30.31, ap=1.21
80 1529431 -57.78 PDB		ChemMol:44019438 NCI Plated 2007:27428 Princeton BioMolecular Research:OSSL_297959 Scientific Exchange (make on demand):M-982072 Aldrich CPR:S786659|ALDRICH TimTec:ST012416 Molport:MolPort-003-917-916 eMolecules:1940342 ...	Mwt: 189.215 xLogP: -2.93 Charge: 0 RotBond: 6 # Protomers: 1 Contact: 1 ES: -74.94 VdW: -19.87 Desolv: p=32.51, ap=4.53	85 901694 -57.60 PDB		Alfa-Aesar:A15408 Apollo Scientific:OR28169 Alfa-Aesar:L10093 Labotest:LT03328588 TimTec Building Blocks:SBB003640 BioSynth:D-1080 Labotest:LT03379865 Bosche Scientific:D14772 ...	Mwt: 119.144 xLogP: -3.96 Charge: 1 RotBond: 3 # Protomers: 3 Contact: 1 ES: -91.31 VdW: -6.55 Desolv: p=38.98, ap=1.27
81 895989 -57.69 PDB		Not for sale	Mwt: 176.148 xLogP: -3.72 Charge: -1 RotBond: 4 # Protomers: 1 Contact: 2 ES: -102.69 VdW: 0.25 Desolv: p=42.25, ap=2.50	86 1532201 -57.52 PDB		Alfa-Aesar:A13154 NCI Plated 2007:97927 NCI Plated 2007:751 Scientific Exchange (make on demand):F-182705 BioSynth:E-4500 Enamine BB Make on Demand:BBV-32844193 Toronto Research Chemicals:E890430 Vitas-M:STK378903 ...	Mwt: 163.242 xLogP: -1.86 Charge: 0 RotBond: 5 # Protomers: 2 Contact: 1 ES: -68.61 VdW: -13.73 Desolv: p=26.47, ap=-1.65
82 30725114 -57.69 PDB		Not for sale	Mwt: 197.131 xLogP: -1.85 Charge: 0 RotBond: 6 # Protomers: 1 Contact: 1 ES: -78.25 VdW: -9.04 Desolv: p=29.73, ap=-0.13	87 4096224 -57.49 PDB		Sigma Aldrich (Building Blocks):A2627|SIGMA Toronto Research Chemicals:A281800	Mwt: 345.232 xLogP: -1.82 Charge: -1 RotBond: 4 # Protomers: 3 Contact: 5 ES: -56.13 VdW: -36.03 Desolv: p=28.00, ap=6.68

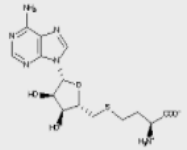
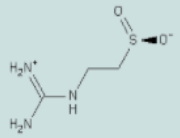
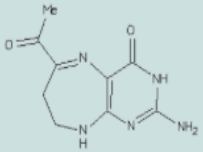
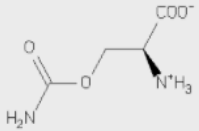
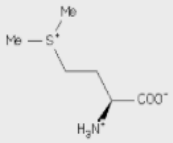
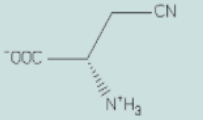
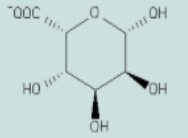
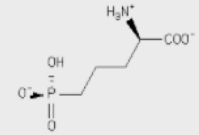
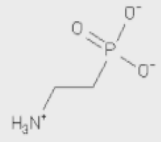
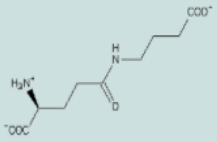
88 693 -57.36 PDB		Labotest:LT00440859 ChemMol:44019548 TimTec Building Blocks:SBB015064 Scientific Exchange (make on demand):F-138384 Vitas-M:STK272180 BioSynth:M-5185 Toronto Research Chemicals:M332685 Aldrich CPR:S783218|ALDRICH ...	Mwt: 195.218 xLogP: -1.25 Charge: 0 RotBond: 3 # Protomers: 1 Contact: 3 ES: -67.83 VdW: -11.95 Desolv: p=23.36, ap=-0.93	93 4096063 -57.14 PDB		Not for sale	Mwt: 312.238 xLogP: -1.51 Charge: 0 RotBond: 4 # Protomers: 3 Contact: 5 ES: -68.51 VdW: -26.06 Desolv: p=36.42, ap=1.00
89 1529313 -57.30 PDB		Maybridge Building Blocks:SB01192 Alfa-Aesar:B20273 Sigma Aldrich (Building Blocks):441597|ALDRICH Sigma Aldrich (Building Blocks):219118|ALDRICH Apollo Scientific:OR26751 Labotest:LT03210177 Sigma Aldrich (Building Blocks):A8131|SIGMA Alfa-Aesar:B24556 ...	Mwt: 132.119 xLogP: -2.81 Charge: 0 RotBond: 3 # Protomers: 2 Contact: 1 ES: -73.08 VdW: -6.74 Desolv: p=20.78, ap=1.75	94 5955041 -57.11 PDB		Not for sale	Mwt: 256.262 xLogP: -5.03 Charge: 0 RotBond: 7 # Protomers: 2 Contact: 3 ES: -96.34 VdW: -17.04 Desolv: p=55.42, ap=0.84
90 4096869 -57.30 PDB		Labotest:LT00249171	Mwt: 140.055 xLogP: -2.80 Charge: -1 RotBond: 2 # Protomers: 1 Contact: 2 ES: -100.36 VdW: -6.26 Desolv: p=45.83, ap=3.49	95 3872987 -57.03 PDB		Ambinter Natural Products:Ambmdy02300228 MicroSource World Drugs:02300228 MicroSource Pharmakon:02300228	Mwt: 212.225 xLogP: -0.81 Charge: -1 RotBond: 4 # Protomers: 1 Contact: 2 ES: -82.51 VdW: -20.77 Desolv: p=48.99, ap=-2.76
91 18181336 -57.28 PDB		Toronto Research Chemicals:D448552 Toronto Research Chemicals:D448550 Cayman Chemical:81882 Molport BB:MolPort-009-018-800	Mwt: 240.243 xLogP: -1.07 Charge: 1 RotBond: 2 # Protomers: 5 Contact: 2 ES: -52.21 VdW: -18.06 Desolv: p=10.53, ap=2.45	96 4228265 -57.02 PDB		Sigma Aldrich (Building Blocks):D7006|SIGMA Sigma Aldrich (Building Blocks):271462|ALDRICH Ambinter Natural Products:GPL000228 FineTech:FT-0624957 TimTec:ST057258 American Custom Chemicals Corp.:VIT0000117	Mwt: 441.404 xLogP: -2.50 Charge: -2 RotBond: 9 # Protomers: 1 Contact: 2 ES: -42.27 VdW: -25.61 Desolv: p=10.81, ap=0.05
92 1529480 -57.18 PDB		Sigma Aldrich (Building Blocks):609234|ALDRICH Indofine:04-1034 ChemMol:30114532 Oakwood Chemical:209256 ChemMol:30113611 AK Scientific:W6670 Sigma Aldrich (Building Blocks):C9517|SIGMA Sigma Aldrich (Building Blocks):C9033|SIGMA ...	Mwt: 123.539 xLogP: -2.43 Charge: 0 RotBond: 2 # Protomers: 2 Contact: 1 ES: -70.93 VdW: -7.49 Desolv: p=21.51, ap=-0.27	97 56509 -56.98 PDB		Acros Organics:35824 Prestwick Chemical:100 Asinex:BAS00319017 Toronto Research Chemicals:M330350 IBScreen:STOCK1S-28836 Sigma Aldrich (Building Blocks):M1404|SIGMA ChemBridge:5213896 eMolecules:717288 ...	Mwt: 301.327 xLogP: 2.96 Charge: 0 RotBond: 3 # Protomers: 2 Contact: 3 ES: -32.66 VdW: -28.74 Desolv: p=7.25, ap=-2.84

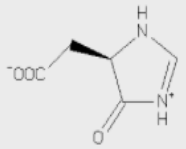
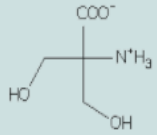
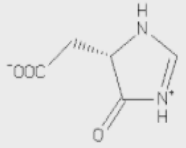
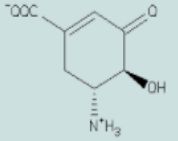
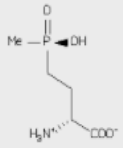
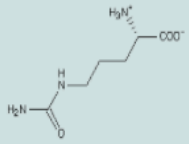
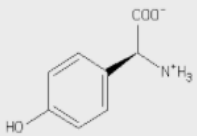
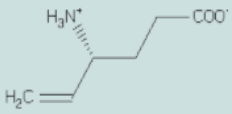
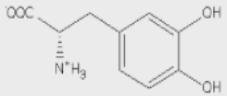
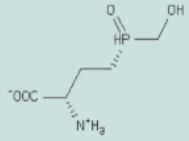
98 4097495 -56.96 PDB		Molport:MolPort-021-804-608 Sigma Aldrich (Building Blocks):74264|SIGMA Ambinter Natural Products:GPN001032 eMolecules:43030631 Ambinter Natural Products:Ambmdy01505190 Indofine:027253S Ambinter Natural Products:NP-009885 BioSynth:Q-100640 ...	Mwt: 177.225 xLogP: -3.39 Charge: 0 RotBond: 5 # Protomers: 1 Contact: 1 ES: -78.93 VdW: -13.57 Desolv: p=33.09, ap=2.45
99 1529356 -56.95 PDB		Not for sale	Mwt: 133.107 xLogP: -2.00 Charge: 0 RotBond: 2 # Protomers: 1 Contact: 1 ES: -76.66 VdW: -11.43 Desolv: p=26.12, ap=5.03
100 21297308 -56.82 PDB		Fluorochem:079649 ChemMol:30102871 BePharm Building Blocks:B17455 ChemMol:30102870 FineTech:FT-0601785 Bosche Scientific:A2312 Oakwood Chemical:079649 ChemMol:44002298 ...	Mwt: 272.181 xLogP: -0.98 Charge: -1 RotBond: 5 # Protomers: 1 Contact: 2 ES: -52.36 VdW: -28.71 Desolv: p=24.68, ap=-0.43
101 56874453 -56.68 PDB		Not for sale	Mwt: 232.369 xLogP: 0.56 Charge: -1 RotBond: 10 # Protomers: 2 Contact: 1 ES: -67.73 VdW: -11.00 Desolv: p=26.26, ap=-4.21
102 1532798 -56.67 PDB		NCI Plated 2007:46994 Alfa-Aesar:L12717 Alfa-Aesar:L13924 Aronis:M07064 Aronis (Make on Request):ASKVIRT/12541 TimTec Building Blocks:SBB000133 Life Chemicals:F3146-3326 BioSynth:A-4920 ...	Mwt: 160.149 xLogP: -2.74 Charge: -1 RotBond: 5 # Protomers: 1 Contact: 1 ES: -87.07 VdW: -10.14 Desolv: p=41.84, ap=-1.30
103 1529200 -56.60 PDB		Vitas-M:STL163324 Labotest:LTBB002768 Princeton BioMolecular Research:OSSL_298270 IBScreen BuildingBlocks:BB_NC-2350 Innovapharm BB Make on Demand:BBV-00053748 BioSynth:C-9760 Sigma Aldrich (Building Blocks):C8630|SIGMA Sigma Aldrich (Building Blocks):285463|ALDRICH ...	Mwt: 240.306 xLogP: -4.86 Charge: 0 RotBond: 7 # Protomers: 3 Contact: 1 ES: -79.86 VdW: -17.81 Desolv: p=42.00, ap=-0.93
104 1530210 -56.48 PDB		Not for sale	Mwt: 174.132 xLogP: -1.45 Charge: -1 RotBond: 5 # Protomers: 1 Contact: 1 ES: -95.00 VdW: -11.78 Desolv: p=48.52, ap=1.78
105 5339228 -56.42 PDB		ChemMol:44012783 Anward:ANW-63651 BePharm Building Blocks:B151243 Enamine BB Make on Demand:BBV-40125462 Ark Pharm Building Blocks:AK-78703 ChemMol:44013397 UORSY BB Make-on-demand:BBV-40125462 Enamine BB Make on Demand:BBV-38275888 ...	Mwt: 151.187 xLogP: -0.71 Charge: 0 RotBond: 3 # Protomers: 1 Contact: 1 ES: -77.85 VdW: -6.62 Desolv: p=25.41, ap=2.64
106 16343242 -56.26 PDB		Not for sale	Mwt: 189.195 xLogP: -3.70 Charge: -1 RotBond: 6 # Protomers: 4 Contact: 2 ES: -67.78 VdW: -16.91 Desolv: p=27.72, ap=0.71
107 30725436 -56.26 PDB		AK Scientific:P546 Molport BB:MolPort-023-220-478	Mwt: 181.128 xLogP: -4.89 Charge: 0 RotBond: 5 # Protomers: 1 Contact: 2 ES: -82.48 VdW: -16.75 Desolv: p=40.16, ap=2.81

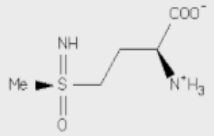
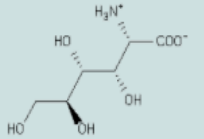
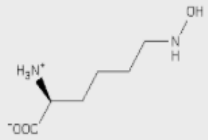
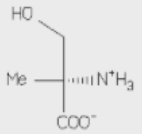
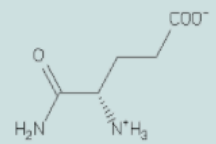
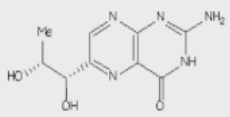
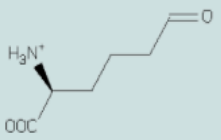
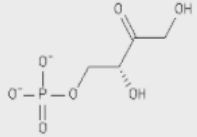
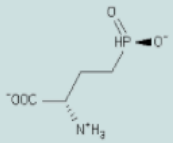
108 1530091 -56.25 PDB		Bosche Scientific:G6326 APIChem:AC-2131	Mwt: 161.157 xLogP: -1.18 Charge: 0 RotBond: 5 # Protomers: 1 Contact: 1 ES: -86.81 VdW: -12.31 Desolv: p=41.68, ap=0.98
109 56874899 -56.20 PDB		Not for sale	Mwt: 176.217 xLogP: -2.09 Charge: -1 RotBond: 5 # Protomers: 2 Contact: 1 ES: -79.31 VdW: -5.93 Desolv: p=30.06, ap=-1.02
110 56871030 -56.18 PDB		Not for sale	Mwt: 132.163 xLogP: -3.56 Charge: 0 RotBond: 3 # Protomers: 1 Contact: 1 ES: -82.42 VdW: -8.57 Desolv: p=34.08, ap=0.73
111 1529886 -56.14 PDB		American Custom Chemicals Corp.:AAA0006233 Ark Pharm Building Blocks:AK128301 AK Scientific:Y0067 Molport BB:MolPort-022-372-684 Molport BB:MolPort-027-947-860	Mwt: 175.12 xLogP: -3.62 Charge: -1 RotBond: 4 # Protomers: 2 Contact: 1 ES: -95.16 VdW: -9.67 Desolv: p=45.82, ap=2.87
112 895960 -56.10 PDB		Not for sale	Mwt: 147.13 xLogP: -3.59 Charge: 0 RotBond: 4 # Protomers: 1 Contact: 2 ES: -70.75 VdW: -13.69 Desolv: p=26.00, ap=2.34
113 897450 -55.98 PDB		NCI Plated 2007:68748	Mwt: 182.183 xLogP: -3.11 Charge: 0 RotBond: 3 # Protomers: 1 Contact: 2 ES: -70.23 VdW: -15.19 Desolv: p=26.06, ap=3.38
114 1532529 -55.96 PDB		Sigma Aldrich (Building Blocks):299154|ALDRICH Sigma Aldrich (Building Blocks):W330108|ALDRICH Sigma Aldrich (Building Blocks):M8439|SIGMA Alfa-Aesar:A10318 Sigma Aldrich (Building Blocks):721271|ALDRICH Sigma Aldrich (Building Blocks):609242|ALDRICH Sigma Aldrich (Building Blocks):64340|FLUKA Alfa-Aesar:A11457 ...	Mwt: 149.215 xLogP: -2.24 Charge: 0 RotBond: 4 # Protomers: 2 Contact: 1 ES: -68.36 VdW: -13.16 Desolv: p=26.68, ap=-1.12
115 39565999 -55.76 PDB		Sigma Aldrich (Building Blocks):M4154|SIGMA Ambinter Natural Products:GPN001250 Toronto Research Chemicals:M285550 Matrix Scientific:057768 ChemMol:97601808 ChemMol:96705798 ChemMol:96705416 AK Scientific:Q369 ...	Mwt: 118.136 xLogP: -3.25 Charge: 0 RotBond: 3 # Protomers: 4 Contact: 1 ES: -77.30 VdW: -5.27 Desolv: p=26.11, ap=0.71
116 55157330 -55.70 PDB		Sigma Aldrich (Building Blocks):268674|ALDRICH Sigma Aldrich (Building Blocks):A6037|SIGMA Ambinter Natural Products:GPL000338 TimTec Make-on-Demand:ST51038107 Ark Pharm Building Blocks:AK113300 BePharm Building Blocks:B70789 Acros Organics:29163 Labotest:LT03331839 ...	Mwt: 125.064 xLogP: -2.14 Charge: 0 RotBond: 2 # Protomers: 1 Contact: 2 ES: -79.01 VdW: -5.34 Desolv: p=26.60, ap=2.05
117 1532838 -55.67 PDB		FineTech:FT-0627825 Alfa-Aesar:B24859 Tractus:RT-014809 American Custom Chemicals Corp.:AAA0000657 American Custom Chemicals Corp.:AAA0004665 Toronto Research Chemicals:A178300	Mwt: 161.157 xLogP: -2.69 Charge: 0 RotBond: 5 # Protomers: 1 Contact: 1 ES: -74.95 VdW: -10.24 Desolv: p=28.61, ap=0.91

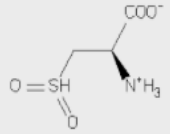
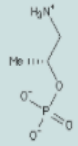
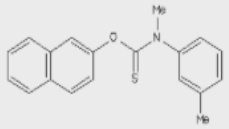
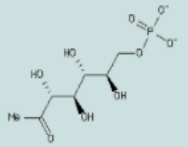
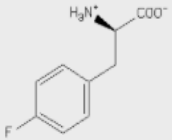
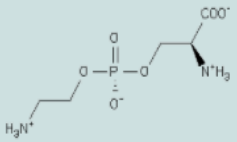
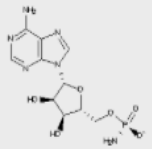
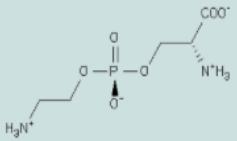
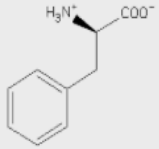
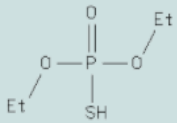
118 1530093 -55.64 PDB		Not for sale	Mwt: 253.175 xLogP: -4.43 Charge: -1 RotBond: 8 # Protomers: 1 Contact: 1 ES: -120.52 VdW: -11.75 Desolv: p=75.96, ap=0.67
119 1530302 -55.51 PDB		Not for sale	Mwt: 147.13 xLogP: -2.84 Charge: 0 RotBond: 4 # Protomers: 1 Contact: 2 ES: -94.75 VdW: -10.06 Desolv: p=46.21, ap=3.09
120 56870945 -55.44 PDB		Not for sale	Mwt: 173.172 xLogP: -3.36 Charge: 0 RotBond: 4 # Protomers: 1 Contact: 1 ES: -73.95 VdW: -1.76 Desolv: p=21.55, ap=-1.28
121 3861771 -55.41 PDB		NCI Plated 2007:15387 Innovapharm Building Blocks:BBS-00012287 Molport:MolPort-003-876-437 Princeton BioMolecular Research:OSSL_379685 BioSynth:M-3585 Enamine BB Make on Demand:BBV-34552597 Toronto Research Chemicals:M294657 TimTec Make-on-Demand:ST50307176 ...	Mwt: 135.188 xLogP: -2.51 Charge: 0 RotBond: 3 # Protomers: 1 Contact: 1 ES: -75.18 VdW: -5.86 Desolv: p=24.00, ap=1.62
122 895314 -55.36 PDB		Pharmeks:PHAR044823 Sigma Aldrich (Building Blocks):615676|ALDRICH Vitas-M:STL164350 Alfa-Aesar:L16281 Innovapharm BB Make on Demand:BBV-00006742 NCI Plated 2007:1901 Enamine BB Make on Demand:BBV-34552812 Toronto Research Chemicals:G821250 ...	Mwt: 117.108 xLogP: -2.49 Charge: 0 RotBond: 3 # Protomers: 1 Contact: 1 ES: -77.19 VdW: -9.75 Desolv: p=28.74, ap=2.84
123 901823 -55.33 PDB		Not for sale	Mwt: 131.131 xLogP: -1.22 Charge: 0 RotBond: 4 # Protomers: 1 Contact: 1 ES: -86.69 VdW: -10.27 Desolv: p=40.20, ap=1.44
124 3873029 -55.32 PDB		Apollo Scientific:OR10671 IBScreen Bioactives:Bio-0618 Acros Organics:32899	Mwt: 167.057 xLogP: -4.26 Charge: -2 RotBond: 3 # Protomers: 2 Contact: 2 ES: -80.25 VdW: -6.27 Desolv: p=29.91, ap=1.29
125 24609815 -55.32 PDB		Not for sale	Mwt: 248.151 xLogP: -1.21 Charge: -1 RotBond: 4 # Protomers: 4 Contact: 4 ES: -67.58 VdW: -19.92 Desolv: p=29.61, ap=2.57
126 4096144 -55.31 PDB		Not for sale	Mwt: 267.303 xLogP: -3.40 Charge: 0 RotBond: 6 # Protomers: 1 Contact: 5 ES: -74.13 VdW: -20.70 Desolv: p=32.57, ap=6.95
127 56871231 -55.27 PDB		Not for sale	Mwt: 289.153 xLogP: -4.46 Charge: -1 RotBond: 8 # Protomers: 2 Contact: 7 ES: -69.70 VdW: -22.25 Desolv: p=30.52, ap=6.17

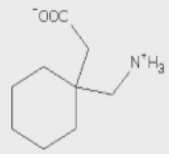
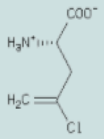
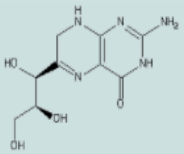
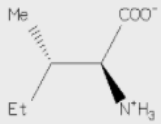
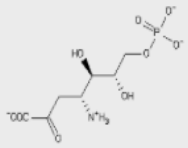
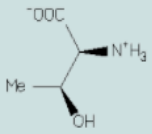
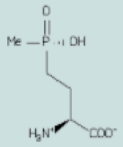
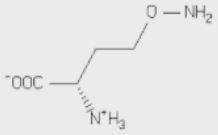
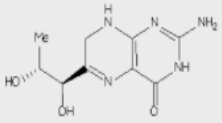
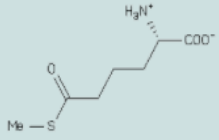
128 4544869 -55.25 PDB		Mcule:MCULE-2578964021	Mwt: 195.171 xLogP: -4.87 Charge: 0 RotBond: 5 # Protomers: 1 Contact: 5 ES: -72.69 VdW: -13.92 Desolv: p=25.93, ap=5.43	
129 895446 -55.15 PDB		Apollo Scientific:OR22613 Sigma Aldrich (Building Blocks):A6262|SIGMA Labotest:LT03328311 Ambinter Natural Products:GPL000153 TimTec Make-on-Demand:ST51037139 Indofine:04-099 ChemMol:30114150 Ambinter:Amb17593668 ...	Mwt: 147.13 xLogP: -2.96 Charge: 0 RotBond: 4 # Protomers: 1 Contact: 1 ES: -77.02 VdW: -7.84 Desolv: p=28.53, ap=1.18	
130 895231 -55.10 PDB		Ark Pharm Building Blocks:AK130678	Mwt: 117.104 xLogP: -2.94 Charge: 0 RotBond: 3 # Protomers: 1 Contact: 1 ES: -77.47 VdW: -7.41 Desolv: p=28.28, ap=1.51	
131 1530209 -55.10 PDB		Not for sale	Mwt: 174.132 xLogP: -1.45 Charge: -1 RotBond: 5 # Protomers: 1 Contact: 1 ES: -97.69 VdW: -6.76 Desolv: p=47.65, ap=1.71	
132 3798750 -54.96 PDB		Prestwick Chemical:501 Sigma Aldrich (Building Blocks):V6261|SIGMA Sigma Aldrich (Building Blocks):V113|SIGMA Ambinter Natural Products:GPS007559 Fluorochem:M02662 Cayman Chemical:9000976 Prestwick Chemical:Prestw-501 Ambinter Natural Products:Ambmdy01502036 ...	Mwt: 129.159 xLogP: -0.39 Charge: 0 RotBond: 4 # Protomers: 1 Contact: 1 ES: -82.64 VdW: -9.85 Desolv: p=38.68, ap=-1.14	
133 113778 -54.94 PDB		Ubichem:51-65-0 PepTech:AL021-1 Apollo Scientific:PC6759 Apollo Scientific:PC4149 Sigma Aldrich (Building Blocks):F4646|SIGMA Alfa-Aesar:L07585 Alfa-Aesar:L19934 Labotest:LT03497133 ...	Mwt: 183.182 xLogP: -1.07 Charge: 0 RotBond: 3 # Protomers: 1 Contact: 2 ES: -62.32 VdW: -14.93 Desolv: p=24.49, ap=-2.18	
134 1532610 -54.92 PDB		Sigma Aldrich (Building Blocks):861839|ALDRICH Aldrich CPR:R415804|ALDRICH Sigma Aldrich (Building Blocks):C9758|SIGMA Labotest:LT03328506 NCI Plated 2007:8921 Sigma Aldrich (Building Blocks):C1625|SIGMA TimTec Make-on-Demand:ST50411688 Indofine:025516 ...	Mwt: 177.184 xLogP: -3.94 Charge: 1 RotBond: 6 # Protomers: 2 Contact: 2 ES: -74.18 VdW: -11.13 Desolv: p=30.02, ap=0.37	
135 895034 -54.87 PDB		Sigma Aldrich (Building Blocks):485985|ALDRICH Sigma Aldrich (Building Blocks):S1315|SIGMA Alfa-Aesar:A11179 Sigma Aldrich (Building Blocks):604887|ALDRICH Sigma Aldrich (Building Blocks):608130|ALDRICH Sigma Aldrich (Building Blocks):78682|FLUKA Sigma Aldrich (Building Blocks):S9771|SIGMA Vitas-M:STK125543 ...	Mwt: 104.085 xLogP: -3.67 Charge: -1 RotBond: 2 # Protomers: 2 Contact: 2 ES: -73.64 VdW: -6.46 Desolv: p=23.32, ap=1.92	
136 1531037 -54.82 PDB		Apollo Scientific:OR1200T NCI Plated 2007:407273 BioSynth:A-4650 FineTech:FT-0627663 Sequoia Research Products:1483-07-4 Sigma Aldrich (Building Blocks):MAR000036|ALDRICH Aldrich CPR:MAR000036|ALDRICH Bachem:F-3670 ...	Mwt: 147.134 xLogP: -3.97 Charge: 0 RotBond: 3 # Protomers: 2 Contact: 1 ES: -74.51 VdW: -12.78 Desolv: p=30.16, ap=2.31	
137 1532820 -54.81 PDB		NCI Plated 2007:521717 Labotest:LT00772005 TimTec Make-on-Demand:ST51015011 Indofine:04-1748 Sigma Aldrich (Building Blocks):G6503|SIGMA Chembo Pharma:KB-38942	Mwt: 145.162 xLogP: -1.04 Charge: 0 RotBond: 5 # Protomers: 1 Contact: 1 ES: -73.70 VdW: -10.82 Desolv: p=30.69, ap=-0.97	

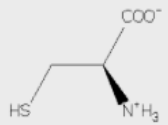
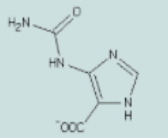
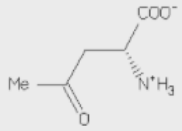
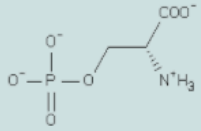
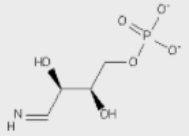
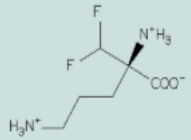
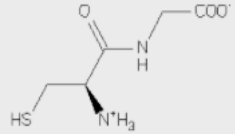
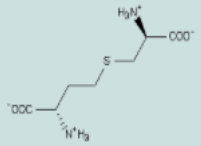
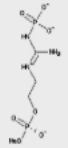
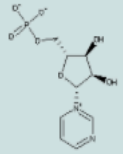
138 4228232 -54.67 PDB		Sigma Aldrich (Building Blocks):A9384|SIGMA Ambinter Natural Products:GPL000150 Molport BB:MolPort-003-925-198 Toronto Research Chemicals:A291500 AK Scientific:X6831	Mwt: 384.418 xLogP: -2.77 Charge: 0 RotBond: 7 # Protomers: 1 Contact: 5 ES: -76.81 VdW: -22.15 Desolv: p=37.59, ap=6.70	143 13507950 -54.61 PDB		Not for sale	Mwt: 151.191 xLogP: -3.18 Charge: 0 RotBond: 4 # Protomers: 1 Contact: 1 ES: -74.53 VdW: -10.98 Desolv: p=29.60, ap=1.29
139 13508952 -54.65 PDB		Not for sale	Mwt: 221.22 xLogP: -0.92 Charge: 0 RotBond: 1 # Protomers: 1 Contact: 2 ES: -50.35 VdW: -14.53 Desolv: p=10.74, ap=-0.51	144 1529574 -54.55 PDB		NCI Plated 2007:128373 Aldrich CPR:PH008887|ALDRICH	Mwt: 148.118 xLogP: -3.55 Charge: 0 RotBond: 4 # Protomers: 1 Contact: 1 ES: -74.97 VdW: -12.24 Desolv: p=28.96, ap=3.71
140 4096040 -54.64 PDB		Alfa-Aesar:B21342 Sigma Aldrich (Building Blocks):64382|SIGMA Aldrich CPR:S859427|ALDRICH Toronto Research Chemicals:M260470 Labotest:LT03380801 ChemDiv BuildingBlocks:BB12-2279 Aldrich CPR:S582026|ALDRICH FineTech:FT-0625490 ...	Mwt: 164.25 xLogP: -4.80 Charge: 0 RotBond: 4 # Protomers: 2 Contact: 1 ES: -67.59 VdW: -11.16 Desolv: p=25.52, ap=-1.41	145 901380 -54.53 PDB		Sigma Aldrich (Building Blocks):C9650|SIGMA NCI Plated 2007:529055 Bachem:F-1460 American Custom Chemicals Corp.:AAA0000747 Combi-Blocks:SS-0235	Mwt: 114.104 xLogP: -3.58 Charge: 0 RotBond: 2 # Protomers: 1 Contact: 1 ES: -75.98 VdW: -5.73 Desolv: p=26.19, ap=0.98
141 4097165 -54.64 PDB		FineTech:FT-0624568 Apollo Scientific Bioactives:BIG1005	Mwt: 193.131 xLogP: -2.77 Charge: -1 RotBond: 1 # Protomers: 1 Contact: 5 ES: -70.44 VdW: -13.17 Desolv: p=22.92, ap=6.05	146 19943047 -54.53 PDB		Sigma Aldrich (Building Blocks):A8054|SIGMA Sigma Aldrich (Building Blocks):283967|ALDRICH Sigma Aldrich (Building Blocks):A6553|SIGMA Sigma Aldrich (Building Blocks):09248|FLUKA Fluorochem:M01267 Pharmeks:PHAR177799 Sigma Aldrich (Building Blocks):A5282|SIGMA Molport:MolPort-000-838-070 ...	Mwt: 196.119 xLogP: -3.75 Charge: -1 RotBond: 5 # Protomers: 1 Contact: 2 ES: -84.36 VdW: -8.75 Desolv: p=38.08, ap=0.50
142 3869509 -54.63 PDB		Labotest:LT03331839 Acros Organics:29163	Mwt: 124.056 xLogP: -2.14 Charge: -1 RotBond: 2 # Protomers: 2 Contact: 2 ES: -76.72 VdW: -7.41 Desolv: p=27.26, ap=2.24	147 1611161 -54.52 PDB		Not for sale	Mwt: 231.228 xLogP: -3.60 Charge: -1 RotBond: 8 # Protomers: 1 Contact: 1 ES: -98.52 VdW: -18.44 Desolv: p=59.66, ap=2.77

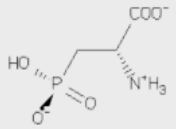
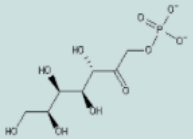
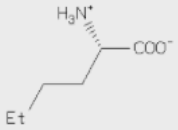
148 30725541 -54.46 PDB		Not for sale	Mwt: 142.114 xLogP: -1.36 Charge: 0 RotBond: 2 # Protomers: 2 Contact: 2 ES: -79.60 VdW: -10.17 Desolv: p=35.84, ap=-0.54		
149 1529583 -54.39 PDB		Not for sale	Mwt: 135.119 xLogP: -2.47 Charge: 0 RotBond: 3 # Protomers: 1 Contact: 3 ES: -83.36 VdW: -1.43 Desolv: p=25.67, ap=4.72		
150 30725537 -54.37 PDB		Not for sale	Mwt: 142.114 xLogP: -1.36 Charge: 0 RotBond: 2 # Protomers: 2 Contact: 2 ES: -75.83 VdW: -11.74 Desolv: p=33.40, ap=-0.20		
151 4102336 -54.26 PDB		Not for sale	Mwt: 171.152 xLogP: -2.46 Charge: 0 RotBond: 1 # Protomers: 1 Contact: 3 ES: -88.91 VdW: -8.33 Desolv: p=39.80, ap=3.17		
152 902217 -54.20 PDB		Not for sale	Mwt: 181.128 xLogP: -3.71 Charge: 0 RotBond: 4 # Protomers: 1 Contact: 2 ES: -79.74 VdW: -9.28 Desolv: p=32.75, ap=2.06		
153 1532614 -54.20 PDB		NCI Plated 2007:46711 Sigma Aldrich (Building Blocks):C83708|ALDRICH Sigma Aldrich (Building Blocks):748935|ALDRICH Alfa-Aesar:A13316 NCI Plated 2007:27425 TimTec Building Blocks:SBB012374 BioSynth:C-6150 Aldrich CPR:S679089|ALDRICH ...	Mwt: 175.188 xLogP: -3.43 Charge: 0 RotBond: 5 # Protomers: 2 Contact: 1 ES: -72.06 VdW: -16.07 Desolv: p=32.17, ap=1.76		
154 6667724 -54.18 PDB		NCI Plated 2007:30081 Molport BB:MolPort-000-005-912 Innovapharm BB Make on Demand:BBV-00053735 Key Organics Building Blocks:FB-0735 Tyger Building Blocks:P16310 Fluorochem:093194 Vitas-M:STK391435 Sigma Aldrich (Building Blocks):56160|ALDRICH ...	Mwt: 167.164 xLogP: -1.81 Charge: 0 RotBond: 2 # Protomers: 1 Contact: 3 ES: -64.24 VdW: -16.17 Desolv: p=26.07, ap=0.16		
155 403618 -54.12 PDB		Prestwick Chemical:501 Sigma Aldrich (Building Blocks):V8261|SIGMA Ambinter Natural Products:GPS007559 Cayman Chemical:9000976 Prestwick Chemical:Prestw-501 Ambinter Natural Products:Ambmdy01502036 Sigma Aldrich (Building Blocks):V022|CERILLIAN Ark Pharm Building Blocks:AK117754 ...	Mwt: 129.159 xLogP: -0.39 Charge: 0 RotBond: 4 # Protomers: 1 Contact: 1 ES: -78.19 VdW: -11.54 Desolv: p=36.65, ap=-1.03		
156 895199 -53.99 PDB		Sigma Aldrich (Building Blocks):102164|ALDRICH Sigma Aldrich (Building Blocks):154318|ALDRICH Alfa-Aesar:41535 Prestwick Chemical:17 Alfa-Aesar:A11311 NCI Plated 2007:16940 Princeton BioMolecular Research:OSSL_298247 Vitas-M:STK801445 ...	Mwt: 197.19 xLogP: -2.20 Charge: 0 RotBond: 3 # Protomers: 1 Contact: 4 ES: -60.85 VdW: -19.54 Desolv: p=25.55, ap=0.85		
157 1530020 -53.99 PDB		AK Scientific:P546 Molport BB:MolPort-023-220-478	Mwt: 181.128 xLogP: -4.36 Charge: 0 RotBond: 5 # Protomers: 1 Contact: 2 ES: -77.99 VdW: -10.29 Desolv: p=31.53, ap=2.76		

158 12366795 -53.99 PDB		Maybridge:JFD01307 NCI Plated 2007:49169 Sigma Aldrich (Building Blocks):76078|SIGMA-ALDRICH Sigma Aldrich (Building Blocks):M9503|SIGMA eMolecules:592359 Toronto Research Chemicals:M260500 Sigma Aldrich (Building Blocks):M5379|SIGMA Prestwick Chemical:523 ...	Mwt: 180.229 xLogP: -3.04 Charge: 0 RotBond: 4 # Protomers: 1 Contact: 2 ES: -71.80 VdW: -13.71 Desolv: p=30.44, ap=1.07
159 13533611 -53.95 PDB		Not for sale	Mwt: 195.171 xLogP: -4.87 Charge: 0 RotBond: 5 # Protomers: 1 Contact: 5 ES: -76.60 VdW: -17.33 Desolv: p=33.90, ap=6.08
160 1532817 -53.90 PDB		Not for sale	Mwt: 162.189 xLogP: -2.52 Charge: 0 RotBond: 6 # Protomers: 1 Contact: 2 ES: -74.21 VdW: -12.34 Desolv: p=29.11, ap=3.54
161 1529425 -53.86 PDB		Alfa-Aesar:A14719 Sigma Aldrich (Building Blocks):07260|FLUKA Prestwick Chemical:960 NCI Plated 2007:26154 TimTec Building Blocks:SBB015068 NCI Plated 2007:400230 ChemBridge:5144149 Vitas-M:STK246894 ...	Mwt: 131.175 xLogP: -0.09 Charge: 0 RotBond: 5 # Protomers: 2 Contact: 1 ES: -87.85 VdW: -7.93 Desolv: p=42.94, ap=-1.02
162 901265 -53.85 PDB		Labotest:LT00772134 Apollo Scientific:OR24937 Sigma Aldrich (Building Blocks):M6877|SIGMA Toronto Research Chemicals:M325970 FineTech:FT-0622265 FineTech:FT-0600572 TimTec Make-on-Demand:ST51015061 ChemMol:30109718 ...	Mwt: 119.12 xLogP: -3.21 Charge: 0 RotBond: 2 # Protomers: 1 Contact: 2 ES: -70.86 VdW: -8.66 Desolv: p=22.93, ap=2.74
163 2508203 -53.81 PDB		UORSY BB Make-on-demand:BBV-39192328 Enamine BB Make on Demand:BBV-39192328 Anward:ANW-63699 BePharm Building Blocks:B136409 Bachem:F-1680 Ark Pharm Building Blocks:AK-76385 Molport BB:MolPort-019-905-197 ...	Mwt: 146.146 xLogP: -1.74 Charge: 0 RotBond: 4 # Protomers: 1 Contact: 1 ES: -88.59 VdW: -10.39 Desolv: p=41.76, ap=3.41
164 17129255 -53.81 PDB		TimTec Building Blocks:SBB067190 Ambinter Natural Products:GPN000331 American Custom Chemicals Corp.:BRS0000332 ChemMol:44031929	Mwt: 237.219 xLogP: -1.46 Charge: 0 RotBond: 2 # Protomers: 1 Contact: 2 ES: -50.49 VdW: -19.21 Desolv: p=13.34, ap=2.55
165 1529204 -53.75 PDB		ChemMol:30102600 Bosche Scientific:A2149 ChemMol:44002130 ChemMol:30102601 APIChem:AC-22380 ChemMol:44002744 Toronto Research Chemicals:O870140 ...	Mwt: 145.158 xLogP: -2.17 Charge: 0 RotBond: 5 # Protomers: 1 Contact: 1 ES: -70.02 VdW: -14.69 Desolv: p=29.72, ap=1.24
166 18206282 -53.73 PDB			Mwt: 197.059 xLogP: -3.09 Charge: -3 RotBond: 5 # Protomers: 6 Contact: 4 ES: -65.26 VdW: -16.15 Desolv: p=24.98, ap=2.69
167 26711216 -53.69 PDB		Not for sale	Mwt: 166.093 xLogP: -4.74 Charge: -1 RotBond: 4 # Protomers: 2 Contact: 1 ES: -98.99 VdW: -4.62 Desolv: p=48.73, ap=1.20

168 4226283 -53.67 PDB		Apollo Scientific:OR9977T	Mwt: 152.151 xLogP: -3.84 Charge: -1 RotBond: 3 # Protomers: 2 Contact: 1 ES: -73.33 VdW: -10.40 Desolv: p=28.24, ap=1.83
169 1529860 -53.64 PDB		Not for sale	Mwt: 154.082 xLogP: -1.82 Charge: -1 RotBond: 3 # Protomers: 1 Contact: 1 ES: -105.93 VdW: -7.84 Desolv: p=58.00, ap=2.13
170 57522 -53.63 PDB		Prestwick Chemical:70 eMolecules:594916 Maybridge:BTB13928 Sigma Aldrich (Building Blocks):T6638|SIGMA Labotest:LT00772359 ChemMol:30104617 FineTech:FT-0603605 Bosche Scientific:T4753 ...	Mwt: 307.418 xLogP: 5.74 Charge: 0 RotBond: 4 # Protomers: 1 Contact: 2 ES: -17.47 VdW: -36.05 Desolv: p=6.49, ap=-6.60
171 56871067 -53.62 PDB		Not for sale	Mwt: 273.154 xLogP: -3.58 Charge: -1 RotBond: 7 # Protomers: 2 Contact: 6 ES: -67.68 VdW: -17.57 Desolv: p=27.39, ap=4.24
172 113781 -53.61 PDB		Ubichem:51-65-0 Sigma Aldrich (Building Blocks):468800|ALDRICH PepTech:AD025-1 Apollo Scientific:PC6759 Apollo Scientific:PC4149 Apollo Scientific:PC0660 Alfa-Aesar:L07585 SynQuest Building Blocks:4757-3-04 ...	Mwt: 183.182 xLogP: -1.07 Charge: 0 RotBond: 3 # Protomers: 1 Contact: 2 ES: -64.22 VdW: -11.20 Desolv: p=23.92, ap=-2.12
173 13351903 -53.61 PDB		Not for sale	Mwt: 228.141 xLogP: -4.81 Charge: 0 RotBond: 7 # Protomers: 1 Contact: 1 ES: -112.00 VdW: -5.78 Desolv: p=61.96, ap=2.21
174 13543487 -53.57 PDB		Sigma Aldrich (Building Blocks):A2627|SIGMA Toronto Research Chemicals:A281800	Mwt: 345.232 xLogP: -1.82 Charge: -1 RotBond: 4 # Protomers: 3 Contact: 5 ES: -59.76 VdW: -28.89 Desolv: p=32.66, ap=2.41
175 4096185 -53.53 PDB		Not for sale	Mwt: 228.141 xLogP: -4.23 Charge: 0 RotBond: 7 # Protomers: 1 Contact: 1 ES: -104.96 VdW: -11.17 Desolv: p=58.58, ap=4.02
176 1927 -53.52 PDB		Maybridge:SB02005 Sigma Aldrich (Building Blocks):299316|ALDRICH Alfa-Aesar:A10132 Sigma Aldrich (Building Blocks):655627|ALDRICH Sigma Aldrich (Building Blocks):589411|ALDRICH Apollo Scientific:OR0702 Alfa-Aesar:A10572 Sigma Aldrich (Building Blocks):78040|FLUKA ...	Mwt: 165.192 xLogP: -1.23 Charge: 0 RotBond: 3 # Protomers: 1 Contact: 2 ES: -63.36 VdW: -10.81 Desolv: p=22.70, ap=-2.06
177 1565280 -53.51 PDB		Sigma Aldrich (Building Blocks):445177|ALDRICH Scientific Exchange:Z-027595 ChemBridge:5116301 NCI Plated 2007:269410 NCI Plated 2007:269409 NCI Plated 2007:269408 NCI Plated 2007:269406 NCI Plated 2007:269405 ...	Mwt: 170.17 xLogP: 1.83 Charge: 0 RotBond: 4 # Protomers: 1 Contact: 2 ES: -51.90 VdW: -11.26 Desolv: p=12.30, ap=-2.65

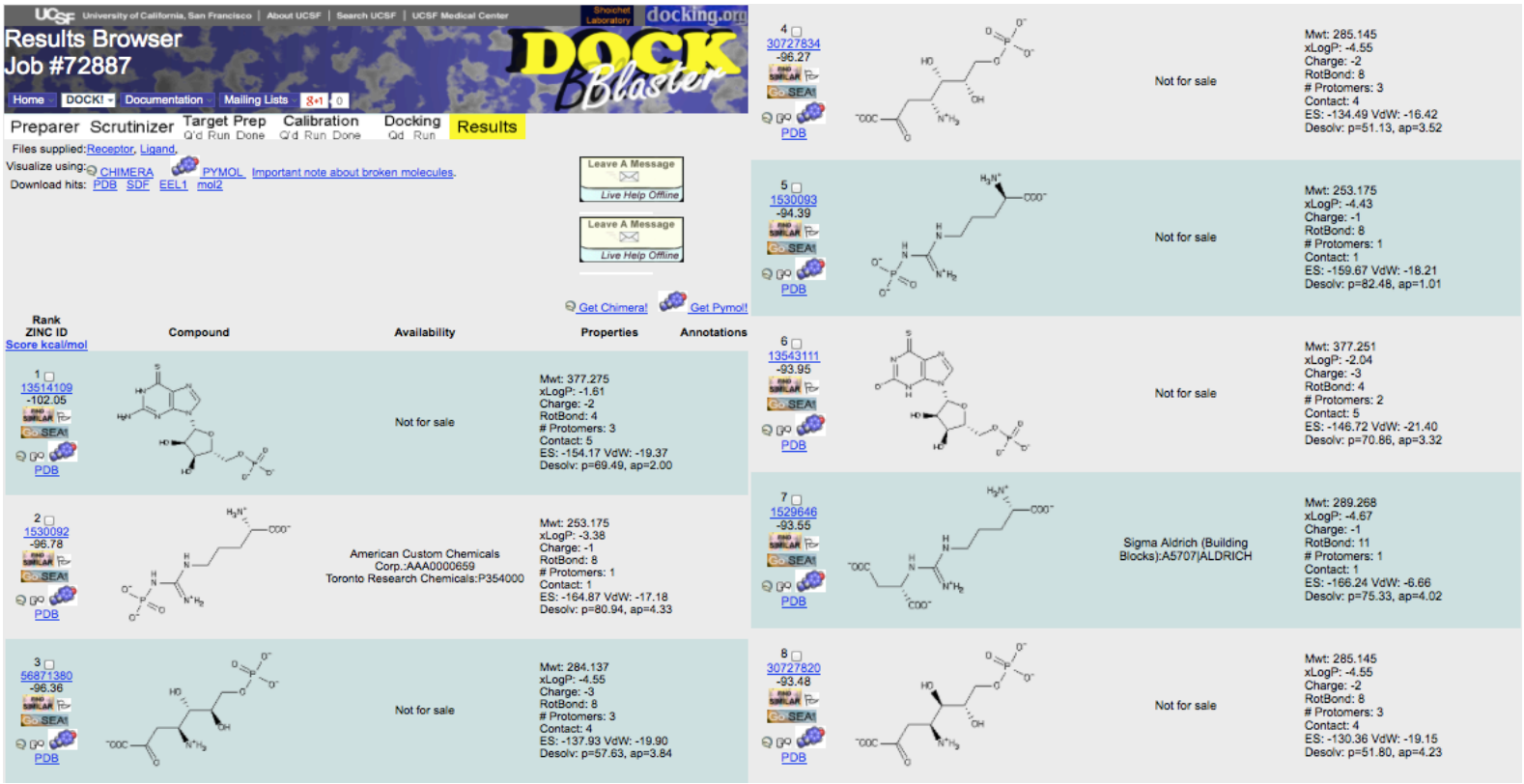
178 4949 -53.50 PDB		Sigma Aldrich (Building Blocks):07748|FLUKA Prestwick Chemical:861 Vitas-M:STK598009 Sigma Aldrich (Building Blocks):G154|SIGMA IBScreen Bioactives:Bio-0045 Toronto Research Chemicals:G117250 Selleck Chemicals:Gabapentin Fluorochem:040287 ...	Mwt: 171.24 xLogP: 0.62 Charge: 0 RotBond: 3 # Protomers: 1 Contact: 3 ES: -71.04 VdW: -11.07 Desolv: p=30.51, ap=-1.89	183 901922 -53.32 PDB		NCI Plated 2007:21943	Mwt: 149.577 xLogP: -1.78 Charge: 0 RotBond: 3 # Protomers: 1 Contact: 1 ES: -74.75 VdW: -7.49 Desolv: p=27.59, ap=1.33
179 4096580 -53.48 PDB			Mwt: 255.234 xLogP: -2.66 Charge: 0 RotBond: 3 # Protomers: 1 Contact: 2 ES: -54.93 VdW: -18.36 Desolv: p=13.25, ap=6.56	184 3581355 -53.27 PDB		Alfa-Aesar:A13699 Sigma Aldrich (Building Blocks):I5281|SIGMA Sigma Aldrich (Building Blocks):PHR1099|FLUKA Sigma Aldrich (Building Blocks):56241|FLUKA Sigma Aldrich (Building Blocks):609013|ALDRICH Labotest:LT03328757 Alfa-Aesar:A17521 Indofine:BIO-227 ...	Mwt: 131.175 xLogP: -1.41 Charge: 0 RotBond: 3 # Protomers: 1 Contact: 1 ES: -67.18 VdW: -10.38 Desolv: p=25.38, ap=-1.08
180 30727824 -53.45 PDB		Not for sale	Mwt: 285.145 xLogP: -4.55 Charge: -2 RotBond: 8 # Protomers: 3 Contact: 4 ES: -102.60 VdW: -11.70 Desolv: p=56.54, ap=4.31	185 1747072 -53.20 PDB		Alfa-Aesar:A10606 Vitas-M:STL163323 Apollo Scientific:OR2477 Scientific Exchange (make on demand):F-415596 OmegaChem:LT-2895 eMolecules:594831 Sigma Aldrich (Building Blocks):05753|FLUKA Sigma Aldrich (Building Blocks):210269|ALDRICH ...	Mwt: 118.112 xLogP: -3.30 Charge: -1 RotBond: 2 # Protomers: 2 Contact: 2 ES: -74.57 VdW: -8.93 Desolv: p=28.55, ap=1.75
181 902209 -53.42 PDB		Not for sale	Mwt: 181.128 xLogP: -3.71 Charge: 0 RotBond: 4 # Protomers: 1 Contact: 2 ES: -69.22 VdW: -16.90 Desolv: p=30.92, ap=1.79	186 1531042 -53.08 PDB		Ambinter:Amb17593409 Enamine Building Blocks:EN300-128726 Anward:ANW-63284 BePharm Building Blocks:B34525 American Custom Chemicals Corp.:CCH0006661 SynQuest Building Blocks:4157-1-08 Ark Pharm Building Blocks:AK-87775 ...	Mwt: 134.135 xLogP: -3.40 Charge: 0 RotBond: 4 # Protomers: 1 Contact: 1 ES: -72.83 VdW: -11.42 Desolv: p=28.38, ap=2.79
182 18155852 -53.38 PDB		Sigma Aldrich (Building Blocks):37272|SIGMA Cayman Chemical:81882 American Custom Chemicals Corp.:BRS0000304 Molport BB:MolPort-009-018-800	Mwt: 240.243 xLogP: -1.65 Charge: 1 RotBond: 2 # Protomers: 3 Contact: 2 ES: -51.04 VdW: -15.82 Desolv: p=10.72, ap=2.77	187 56871174 -53.08 PDB		Not for sale	Mwt: 190.244 xLogP: -1.58 Charge: -1 RotBond: 6 # Protomers: 2 Contact: 1 ES: -60.52 VdW: -16.11 Desolv: p=25.09, ap=-1.54

188 895042 -53.06 PDB		Sigma Aldrich (Building Blocks):W326305|ALDRICH Pharmeks:PHAR092878 Sigma Aldrich (Building Blocks):C121800|ALDRICH Sigma Aldrich (Building Blocks):C5360|SIGMA Alfa-Aesar:A10389 Molcan:acetylcysteine-ep-im Sigma Aldrich (Building Blocks):609129|ALDRICH Sigma Aldrich (Building Blocks):30130|FLUKA ...	Mwt: 121.161 xLogP: -2.71 Charge: 0 RotBond: 2 # Protomers: 2 Contact: 2 ES: -73.03 VdW: -7.15 Desolv: p=24.99, ap=2.13
189 3869343 -53.04 PDB		Not for sale	Mwt: 170.128 xLogP: -1.00 Charge: 0 RotBond: 2 # Protomers: 2 Contact: 3 ES: -65.54 VdW: -16.81 Desolv: p=26.96, ap=2.36
190 901723 -53.03 PDB		UORSY BB Make-on-demand:BBV-44242927 Enamine Building Blocks:EN300-117761 Enamine BB Make on Demand:BBV-44242927	Mwt: 131.131 xLogP: -3.22 Charge: 0 RotBond: 3 # Protomers: 1 Contact: 1 ES: -70.78 VdW: -7.64 Desolv: p=23.93, ap=1.47
191 3869281 -52.93 PDB		Sigma Aldrich (Building Blocks):79710|SIGMA Scientific Exchange (make on demand):M-982071 TimTec Building Blocks:SBB000251 Aldrich CPR:S364827|ALDRICH Labotest:LT00451670 eMolecules:530918 TimTec Make-on-Demand:ST51006697 Indofine:04-2317 ...	Mwt: 184.064 xLogP: -4.29 Charge: -1 RotBond: 4 # Protomers: 2 Contact: 1 ES: -130.54 VdW: -4.47 Desolv: p=81.41, ap=0.67
192 30727889 -52.93 PDB		Not for sale	Mwt: 198.091 xLogP: -2.65 Charge: -1 RotBond: 5 # Protomers: 2 Contact: 5 ES: -63.88 VdW: -17.32 Desolv: p=24.59, ap=3.68
193 1530962 -52.92 PDB		Bosche Scientific:E2777 APIChem:AC-388 APIChem:AC-5497	Mwt: 183.178 xLogP: -3.04 Charge: 1 RotBond: 5 # Protomers: 2 Contact: 1 ES: -89.75 VdW: -0.49 Desolv: p=36.91, ap=0.40
194 4095652 -52.91 PDB		Sigma Aldrich (Building Blocks):C0166|SIGMA Ambinter:Amb17594540 Bachem:G-3755 American Custom Chemicals Corp.:AAA0006195 Chembo Pharma:KB-53176	Mwt: 178.213 xLogP: -3.06 Charge: 0 RotBond: 4 # Protomers: 2 Contact: 2 ES: -86.00 VdW: -5.80 Desolv: p=35.54, ap=3.35
195 1529407 -52.84 PDB		Sigma Aldrich (Building Blocks):C3633|SIGMA Princeton BioMolecular Research:OSSL_297345 Scientific Exchange (make on demand):F-137764 Aldrich CPR:S788988|ALDRICH Ambinter Natural Products:GPL000311 TimTec:ST086627 eMolecules:536031 ChemDiv BuildingBlocks:BB55-7486 ...	Mwt: 222.266 xLogP: -4.46 Charge: 0 RotBond: 7 # Protomers: 1 Contact: 1 ES: -85.81 VdW: -18.27 Desolv: p=47.51, ap=3.72
196 13521326 -52.76 PDB		Not for sale	Mwt: 275.094 xLogP: -2.89 Charge: -2 RotBond: 7 # Protomers: 2 Contact: 2 ES: -89.05 VdW: -16.27 Desolv: p=51.95, ap=0.62
197 30724836 -52.74 PDB		Not for sale	Mwt: 291.176 xLogP: -5.18 Charge: -1 RotBond: 4 # Protomers: 2 Contact: 5 ES: -72.46 VdW: -16.93 Desolv: p=34.80, ap=1.85

198 49538598 -52.74 PDB		Sigma Aldrich (Building Blocks):A4910|SIGMA ZereneX Building Blocks:ZBioX-0490 Fluorochem:M01258 IBScreen Bioactives:Bio-0618 Molport BB:MolPort-001-759-594 Acros Organics:32899 Apollo Scientific:OR10671 Ryan Scientific BB:OR10671 ...	Mwt: 168.065 xLogP: -4.26 Charge: -1 RotBond: 3 # Protomers: 1 Contact: 2 ES: -72.49 VdW: -11.22 Desolv: p=29.61, ap=1.37
199 4097057 -52.73 PDB		Not for sale	Mwt: 288.145 xLogP: -4.46 Charge: -2 RotBond: 8 # Protomers: 2 Contact: 7 ES: -73.76 VdW: -14.40 Desolv: p=29.49, ap=5.95
200 1529322 -52.72 PDB		Labotest:LT03328922 Alfa-Aesar:A10791 Sigma Aldrich (Building Blocks):N8513|SIGMA Alfa-Aesar:L03913 ChemMol:44024386 Scientific Exchange (make on demand):F-415585 OmegaChem:NR-2757 BioSynth:N-8796 ...	Mwt: 131.175 xLogP: -1.09 Charge: 0 RotBond: 4 # Protomers: 1 Contact: 1 ES: -72.11 VdW: -9.53 Desolv: p=27.67, ap=1.25

Results from the docking of 1YCL PDB code: 72887. The re-docking of the SRH onto the LuxS active site showed great results (in green). This run was done using the faster scheme run vs. the slower scheme. Below is the result page that contains 200 potential inhibitors for the LuxS enzyme according the KEGG database. SRH did not re-dock as a result from the KEGG database.

		Scoring	
		Polarized	AMBER
Sampling	Coarser	7.731 / 4	7.612 / 11
	Finer	7.714 / 4	7.611 / 12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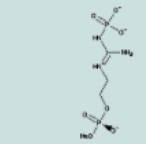
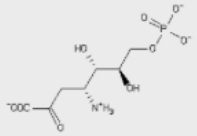
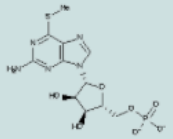
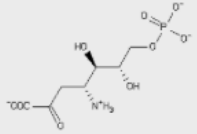
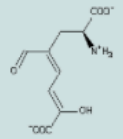
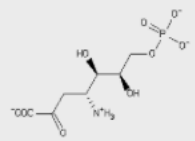
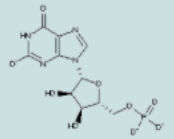
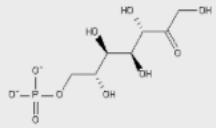
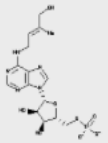
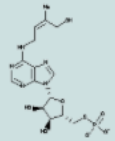
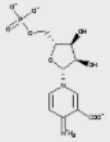
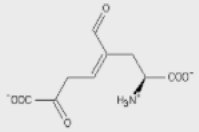
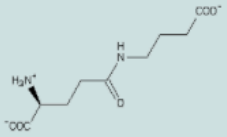
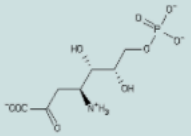
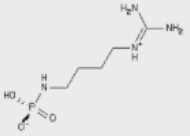
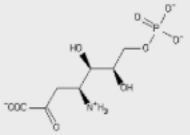
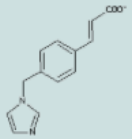
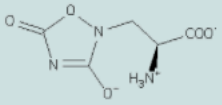
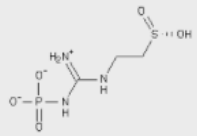
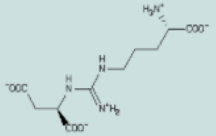
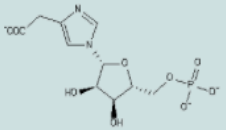
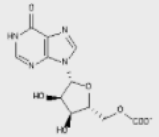
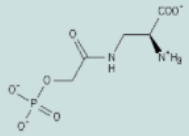
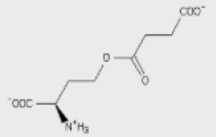
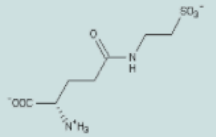
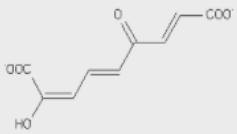
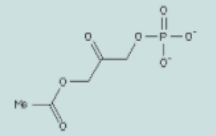
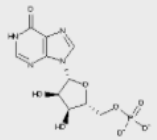
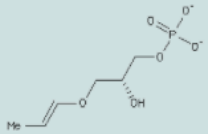
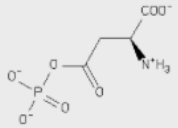
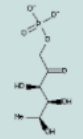
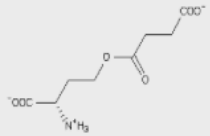
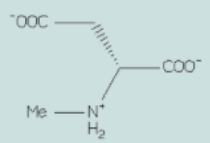
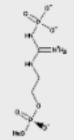
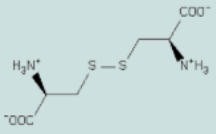
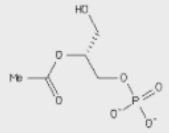
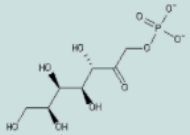
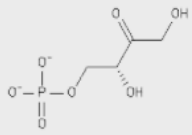
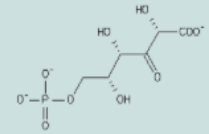
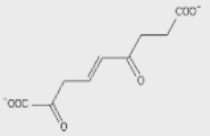
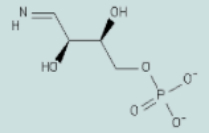
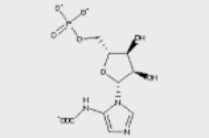
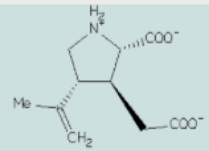
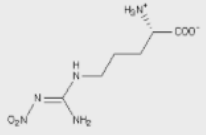
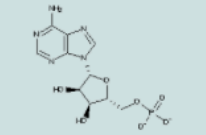
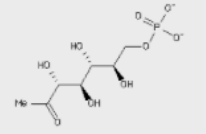
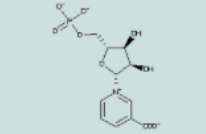
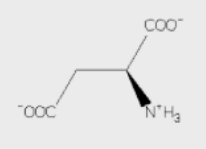
Target Prep
Q'd Run Done
Calibration
Q'd Run Done
Docking
Qd Run
Results
Files supplied: Receptor, Ligand,
Visualize using: CHIMERA PYMOL Important note about broken molecules.
Download hits: PDB SDF EEL1 mol2
Leave A Message
Live Help Offline
Leave A Message
Live Help Offline
Get Chimera!
Get Pymol!
Rank
ZINC ID
Score kcal/mol
Compound
Availability
Properties
Annotations
1
13514109
-102.05
PDB
Not for sale
Mwt: 377.275
xLogP: -1.61
Charge: -2
RotBond: 4
Protomers: 3
Contact: 5
ES: -154.17 VdW: -19.37
Desolv: p=69.49, ap=2.00
2
1530092
-96.78
PDB
American Custom Chemicals Corp.:AAA0000659
Toronto Research Chemicals:P354000
Mwt: 253.175
xLogP: -3.38
Charge: -1
RotBond: 8
Protomers: 1
Contact: 1
ES: -164.87 VdW: -17.18
Desolv: p=80.94, ap=4.33
3
56871380
-96.36
PDB
Not for sale
Mwt: 284.137
xLogP: -4.55
Charge: -3
RotBond: 8
Protomers: 3
Contact: 4
ES: -137.93 VdW: -19.90
Desolv: p=57.63, ap=3.84
4
30727834
-96.27
PDB
Not for sale
Mwt: 285.145
xLogP: -4.55
Charge: -2
RotBond: 8
Protomers: 3
Contact: 4
ES: -134.49 VdW: -16.42
Desolv: p=51.13, ap=3.52
5
1530093
-94.39
PDB
Not for sale
Mwt: 253.175
xLogP: -4.43
Charge: -1
RotBond: 8
Protomers: 1
Contact: 1
ES: -159.67 VdW: -18.21
Desolv: p=82.48, ap=1.01
6
13543111
-93.95
PDB
Not for sale
Mwt: 377.251
xLogP: -2.04
Charge: -3
RotBond: 4
Protomers: 2
Contact: 5
ES: -146.72 VdW: -21.40
Desolv: p=70.86, ap=3.32
7
1529646
-93.55
PDB
Sigma Aldrich (Building Blocks):A5707|ALDRICH
Mwt: 289.268
xLogP: -4.67
Charge: -1
RotBond: 11
Protomers: 1
Contact: 1
ES: -166.24 VdW: -6.66
Desolv: p=75.33, ap=4.02
8
30727820
-93.48
PDB
Not for sale
Mwt: 285.145
xLogP: -4.55
Charge: -2
RotBond: 8
Protomers: 3
Contact: 4
ES: -130.36 VdW: -19.15
Desolv: p=51.80, ap=4.23

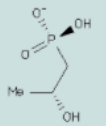
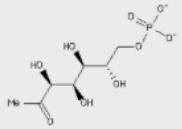
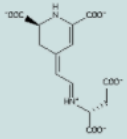
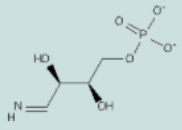
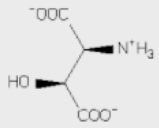
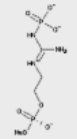
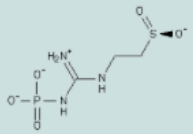
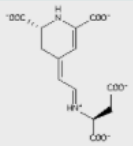
9 31970429 -92.68 PDB		Not for sale	Mwt: 276.102 xLogP: -2.76 Charge: -1 RotBond: 8 # Protomers: 4 Contact: 2 ES: -111.29 VdW: -22.98 Desolv: p=40.82, ap=0.77
10 56871381 -90.91 PDB		Not for sale	Mwt: 284.137 xLogP: -4.55 Charge: -3 RotBond: 8 # Protomers: 3 Contact: 4 ES: -129.49 VdW: -16.81 Desolv: p=52.61, ap=2.79
11 30731290 -89.91 PDB		Not for sale	Mwt: 391.302 xLogP: -1.41 Charge: -2 RotBond: 5 # Protomers: 2 Contact: 5 ES: -115.87 VdW: -16.85 Desolv: p=41.14, ap=1.67
12 30727824 -88.25 PDB		Not for sale	Mwt: 285.145 xLogP: -4.55 Charge: -2 RotBond: 8 # Protomers: 3 Contact: 4 ES: -144.39 VdW: -11.90 Desolv: p=63.53, ap=4.50
13 30725483 -87.86 PDB		Not for sale	Mwt: 227.172 xLogP: -3.06 Charge: -2 RotBond: 6 # Protomers: 3 Contact: 2 ES: -111.77 VdW: -16.28 Desolv: p=40.50, ap=-0.32
14 56871383 -87.65 PDB		Not for sale	Mwt: 284.137 xLogP: -4.55 Charge: -3 RotBond: 8 # Protomers: 3 Contact: 4 ES: -129.36 VdW: -14.54 Desolv: p=53.11, ap=3.13
15 20233084 -86.75 PDB		Sigma Aldrich (Building Blocks):95550|FLUKA Ambinter Natural Products:GPL000125 Sigma Aldrich (Building Blocks):X1000|SIGMA Sequoia Research Products:25899-70-1 Toronto Research Chemicals:X743800 Amadis Chemical:A848951	Mwt: 363.199 xLogP: -2.84 Charge: -1 RotBond: 4 # Protomers: 3 Contact: 5 ES: -134.62 VdW: -25.54 Desolv: p=68.39, ap=5.02
16 56871166 -84.22 PDB		Not for sale	Mwt: 289.153 xLogP: -4.46 Charge: -1 RotBond: 8 # Protomers: 2 Contact: 7 ES: -116.11 VdW: -12.43 Desolv: p=37.62, ap=6.69
17 1586420 -83.30 PDB		Sigma Aldrich (Building Blocks):D5638|SIGMA NCI Plated 2007:8125 eMolecules:485647 Molport:MolPort-003-927-207 AnalytiCon Discovery NP:NP-012103 Cayman Chemical:88820 Ambinter Natural Products:NP-012103 Anward:ANW-34788 ...	Mwt: 226.272 xLogP: 3.13 Charge: -2 RotBond: 10 # Protomers: 1 Contact: 1 ES: -126.63 VdW: -17.72 Desolv: p=61.69, ap=-0.64
18 30730832 -82.34 PDB		Not for sale	Mwt: 429.326 xLogP: -0.71 Charge: -2 RotBond: 8 # Protomers: 2 Contact: 5 ES: -89.33 VdW: -30.32 Desolv: p=35.86, ap=1.44

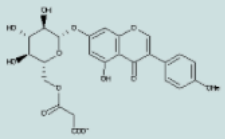
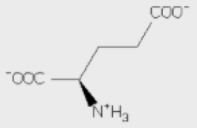
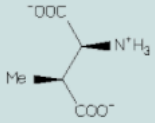
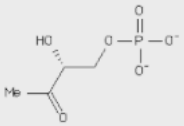
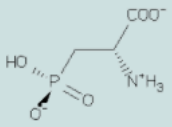
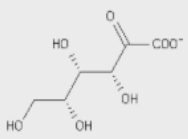
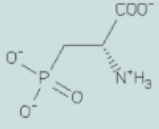
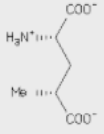
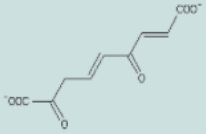
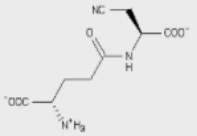
19 30730837 -82.01 PDB		Not for sale	Mwt: 429.326 xLogP: -0.71 Charge: -2 RotBond: 8 # Protomers: 2 Contact: 5 ES: -89.08 VdW: -33.12 Desolv: p=38.09, ap=2.10	24 30726346 -80.97 PDB		Not for sale	Mwt: 348.204 xLogP: -3.27 Charge: -2 RotBond: 5 # Protomers: 2 Contact: 5 ES: -112.56 VdW: -25.15 Desolv: p=53.73, ap=3.01
20 1530053 -81.85 PDB		Not for sale	Mwt: 228.18 xLogP: -3.80 Charge: -1 RotBond: 7 # Protomers: 1 Contact: 1 ES: -106.74 VdW: -22.34 Desolv: p=44.61, ap=2.60	25 1611161 -80.94 PDB		Not for sale	Mwt: 231.228 xLogP: -3.60 Charge: -1 RotBond: 8 # Protomers: 1 Contact: 1 ES: -131.19 VdW: -15.69 Desolv: p=63.12, ap=2.82
21 30727829 -81.82 PDB		Not for sale	Mwt: 285.145 xLogP: -4.55 Charge: -2 RotBond: 8 # Protomers: 3 Contact: 4 ES: -117.95 VdW: -26.98 Desolv: p=59.03, ap=4.08	26 30724479 -80.66 PDB		Not for sale	Mwt: 210.174 xLogP: -1.78 Charge: 0 RotBond: 7 # Protomers: 2 Contact: 2 ES: -110.69 VdW: -11.91 Desolv: p=40.26, ap=1.67
22 56871382 -81.64 PDB		Not for sale	Mwt: 284.137 xLogP: -4.55 Charge: -3 RotBond: 8 # Protomers: 3 Contact: 4 ES: -123.35 VdW: -19.83 Desolv: p=58.77, ap=2.77	27 5389 -80.15 PDB		Prestwick Chemical:979 Sigma Aldrich (Building Blocks):Q1386|SIGMA ChemMol:30104677 FineTech:FT-0602473 Bosche Scientific:O3166 ChemMol:30104678 ChemMol:30109441 ChemMol:97901018 ...	Mwt: 227.243 xLogP: 1.56 Charge: -1 RotBond: 4 # Protomers: 2 Contact: 3 ES: -89.89 VdW: -22.74 Desolv: p=38.17, ap=-5.69
23 13779018 -81.32 PDB		Sigma Aldrich (Building Blocks):Q2128|SIGMA Ambinter Natural Products:GPN000665 FineTech:FT-0604435 Fluorochem:M02436 ChemMol:97900382 TimTec Make-on-Demand:ST50825297 AK Scientific:V0329 Molport BB:MolPort-003-939-212 ...	Mwt: 187.111 xLogP: -4.04 Charge: -2 RotBond: 3 # Protomers: 3 Contact: 2 ES: -90.35 VdW: -16.46 Desolv: p=22.40, ap=3.10	28 15265482 -79.93 PDB		Not for sale	Mwt: 230.162 xLogP: -4.08 Charge: -1 RotBond: 6 # Protomers: 2 Contact: 2 ES: -129.50 VdW: -18.24 Desolv: p=65.59, ap=2.23

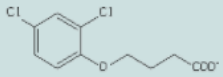
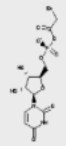
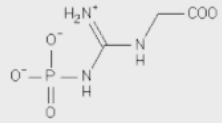
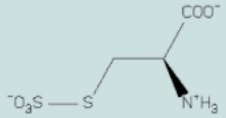
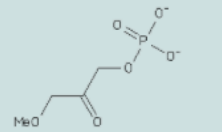
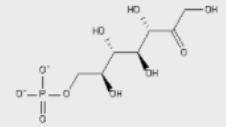
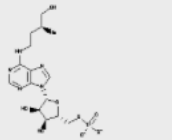
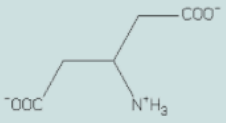
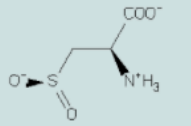
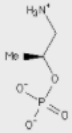
29 1529647 -79.77 PDB		Not for sale	Mwt: 289.268 xLogP: -4.67 Charge: -1 RotBond: 11 # Protomers: 1 Contact: 1 ES: -155.48 VdW: -2.71 Desolv: p=74.38, ap=4.04
30 1531045 -79.68 PDB		NCI Plated 2007:97405 NCI Plated 2007:76960 ChemBridge:5163230 Sigma Aldrich (Building Blocks):615617|ALDRICH Alfa-Aesar:A14158 NCI Plated 2007:19492 Aldrich CPR:S449512|ALDRICH Sigma Aldrich (Building Blocks):84810|FLUKA ...	Mwt: 200.234 xLogP: 2.38 Charge: -2 RotBond: 9 # Protomers: 2 Contact: 1 ES: -121.87 VdW: -17.42 Desolv: p=64.47, ap=-4.87
31 4096415 -79.57 PDB		Not for sale	Mwt: 336.193 xLogP: -2.17 Charge: -2 RotBond: 6 # Protomers: 1 Contact: 5 ES: -146.44 VdW: -31.45 Desolv: p=94.62, ap=3.69
32 4096063 -79.42 PDB		Not for sale	Mwt: 312.238 xLogP: -1.51 Charge: 0 RotBond: 4 # Protomers: 3 Contact: 5 ES: -98.18 VdW: -26.53 Desolv: p=44.54, ap=0.76
33 1529852 -79.21 PDB		Not for sale	Mwt: 240.108 xLogP: -4.21 Charge: -2 RotBond: 6 # Protomers: 1 Contact: 1 ES: -177.68 VdW: -20.11 Desolv: p=115.29, ap=3.29
34 1530265 -79.00 PDB		Not for sale	Mwt: 218.185 xLogP: -3.42 Charge: -1 RotBond: 8 # Protomers: 1 Contact: 1 ES: -116.30 VdW: -18.94 Desolv: p=54.87, ap=1.37
35 1530285 -78.48 PDB		Ark Pharm Building Blocks:AK110174 BePharm Building Blocks:B132133 AK Scientific:Y0426 Molport BB:MolPort-022-332-563	Mwt: 253.256 xLogP: -4.83 Charge: -1 RotBond: 7 # Protomers: 2 Contact: 1 ES: -128.26 VdW: -19.26 Desolv: p=61.43, ap=7.61
36 30728153 -78.16 PDB		Not for sale	Mwt: 210.141 xLogP: -0.80 Charge: -2 RotBond: 5 # Protomers: 1 Contact: 2 ES: -123.12 VdW: -17.07 Desolv: p=62.88, ap=-0.85
37 56870963 -77.79 PDB		Not for sale	Mwt: 211.086 xLogP: -1.75 Charge: -1 RotBond: 6 # Protomers: 2 Contact: 2 ES: -93.41 VdW: -15.36 Desolv: p=31.66, ap=-0.68
38 4228242 -77.75 PDB		TimTec Building Blocks:SBB006471 eMolecules:4796930 Labotest:LT00847433 FineTech:FT-0627236 TimTec:ST057084 IBScreen NP:STOCK1N-29752 Bosche Scientific:I6967 ChemMol:30114362 ...	Mwt: 347.2 xLogP: -2.49 Charge: -1 RotBond: 4 # Protomers: 2 Contact: 5 ES: -94.02 VdW: -27.22 Desolv: p=41.28, ap=2.21

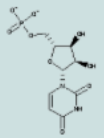
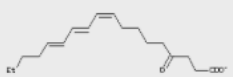
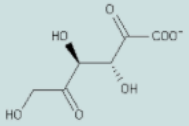
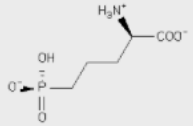
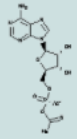
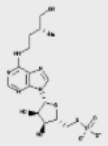
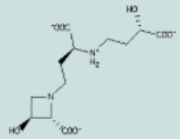
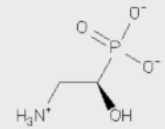
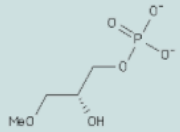
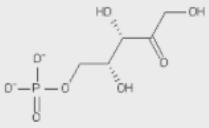
39 56874077 -77.63 PDB		Not for sale	Mwt: 211.13 xLogP: -1.09 Charge: -1 RotBond: 6 # Protomers: 2 Contact: 3 ES: -93.27 VdW: -15.67 Desolv: p=30.36, ap=0.95
40 3870028 -76.88 PDB		Not for sale	Mwt: 211.066 xLogP: -2.31 Charge: -2 RotBond: 5 # Protomers: 1 Contact: 1 ES: -142.76 VdW: -13.49 Desolv: p=77.91, ap=1.47
41 13508310 -76.88 PDB		Not for sale	Mwt: 242.12 xLogP: -3.16 Charge: -2 RotBond: 6 # Protomers: 3 Contact: 5 ES: -92.55 VdW: -20.78 Desolv: p=33.21, ap=3.24
42 1532858 -76.82 PDB		Sigma Aldrich (Building Blocks):S7129|SIGMA ChiralBlock BioScience BB:376 American Custom Chemicals Corp.:AAA0003408 ChemMol:97600130 Apexmol Building Blocks:AM806589	Mwt: 218.185 xLogP: -3.42 Charge: -1 RotBond: 8 # Protomers: 1 Contact: 1 ES: -116.81 VdW: -13.89 Desolv: p=52.67, ap=1.21
43 901012 -76.56 PDB		Aldrich CPR:R426059|ALDRICH Sigma Aldrich (Building Blocks):M2137|SIGMA IBScreen BuildingBlocks:BB_NC-1943 Vitas-M:STK246904 Sigma Aldrich (Building Blocks):65831|FLUKA Sigma Aldrich (Building Blocks):M3262|SIGMA Labotest:LT03328838 FineTech:FT-0602829 ...	Mwt: 146.122 xLogP: -2.61 Charge: -1 RotBond: 4 # Protomers: 2 Contact: 1 ES: -104.96 VdW: -6.78 Desolv: p=36.99, ap=-1.81
44 4096439 -76.40 PDB		Not for sale	Mwt: 275.094 xLogP: -2.76 Charge: -2 RotBond: 8 # Protomers: 1 Contact: 1 ES: -125.48 VdW: -21.80 Desolv: p=70.81, ap=0.07
45 1532673 -76.18 PDB		Alfa-Aesar:A13762 Sigma Aldrich (Building Blocks):C122009|ALDRICH Sigma Aldrich (Building Blocks):C6735|SIGMA Sigma Aldrich (Building Blocks):600105|ALDRICH Sigma Aldrich (Building Blocks):057490|SAJ Molcan:acetylcysteine-ep-im Vitas-M:STL163324 Apollo Scientific:OR0325 ...	Mwt: 240.306 xLogP: -4.86 Charge: 0 RotBond: 7 # Protomers: 3 Contact: 1 ES: -104.03 VdW: -12.61 Desolv: p=41.37, ap=-0.90
46 56871036 -75.93 PDB		Not for sale	Mwt: 213.102 xLogP: -1.56 Charge: -1 RotBond: 6 # Protomers: 2 Contact: 3 ES: -91.84 VdW: -17.74 Desolv: p=32.79, ap=0.85
47 4097057 -75.70 PDB		Not for sale	Mwt: 288.145 xLogP: -4.46 Charge: -2 RotBond: 8 # Protomers: 2 Contact: 7 ES: -92.63 VdW: -22.51 Desolv: p=33.68, ap=5.76
48 18206282 -75.69 PDB			Mwt: 197.059 xLogP: -3.09 Charge: -3 RotBond: 5 # Protomers: 6 Contact: 4 ES: -94.25 VdW: -15.19 Desolv: p=30.80, ap=2.95

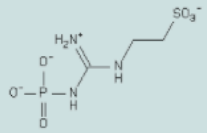
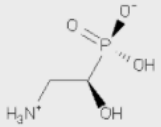
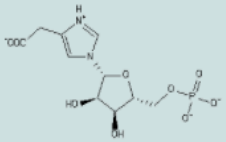
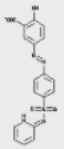
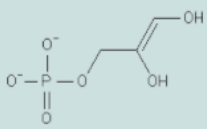
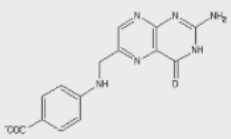
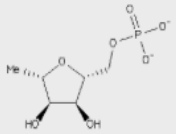
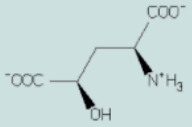
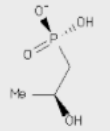
49 31259415 -75.67 PDB		Not for sale	Mwt: 271.094 xLogP: -4.41 Charge: -3 RotBond: 7 # Protomers: 3 Contact: 5 ES: -105.16 VdW: -25.13 Desolv: p=52.07, ap=2.56
50 1529967 -75.40 PDB		Not for sale	Mwt: 212.157 xLogP: -1.53 Charge: -2 RotBond: 7 # Protomers: 1 Contact: 1 ES: -114.33 VdW: -22.26 Desolv: p=61.82, ap=-0.64
51 30727884 -75.37 PDB		Not for sale	Mwt: 198.091 xLogP: -2.65 Charge: -1 RotBond: 5 # Protomers: 2 Contact: 5 ES: -102.09 VdW: -10.36 Desolv: p=32.78, ap=4.30
52 30729492 -75.33 PDB		Not for sale	Mwt: 336.173 xLogP: -2.61 Charge: -3 RotBond: 5 # Protomers: 3 Contact: 5 ES: -121.42 VdW: -25.13 Desolv: p=67.75, ap=3.47
53 1555758 -74.99 PDB		Not for sale	Mwt: 212.225 xLogP: -0.81 Charge: -1 RotBond: 4 # Protomers: 1 Contact: 4 ES: -90.46 VdW: -16.52 Desolv: p=34.65, ap=-2.66
54 19796052 -74.92 PDB		Sigma Aldrich (Building Blocks):72750|FLUKA FineTech:FT-0629730 eMolecules:529712 TimTec Make-on-Demand:ST50975058 Indofine:04-2167 Cayman Chemical:80220 Princeton BioMolecular Research:OSSL_108087 ChemDiv BuildingBlocks:BB55-8569 ...	Mwt: 218.193 xLogP: -3.63 Charge: -1 RotBond: 7 # Protomers: 4 Contact: 1 ES: -100.32 VdW: -15.22 Desolv: p=40.94, ap=-0.31
55 3860156 -74.50 PDB		Sigma Aldrich (Building Blocks):A25008|ALDRICH Prestwick Chemical:356 Sigma Aldrich (Building Blocks):A4659|SIGMA Alfa-Aesar:L14051 TimTec Building Blocks:SBB001403 IBScreen BuildingBlocks:BB_NC-1914 Indofine:BIO-014 Sigma Aldrich (Building Blocks):662658|ALDRICH ...	Mwt: 346.216 xLogP: -1.52 Charge: -1 RotBond: 4 # Protomers: 2 Contact: 5 ES: -84.84 VdW: -26.46 Desolv: p=34.48, ap=2.31
56 56871067 -74.50 PDB		Not for sale	Mwt: 273.154 xLogP: -3.58 Charge: -1 RotBond: 7 # Protomers: 2 Contact: 6 ES: -96.12 VdW: -14.79 Desolv: p=32.57, ap=3.85
57 4095572 -74.14 PDB		Ambinter Natural Products:GPL000249 American Custom Chemicals Corp.:VIT0000120 Toronto Research Chemicals:N429392	Mwt: 333.189 xLogP: -4.59 Charge: -2 RotBond: 5 # Protomers: 1 Contact: 5 ES: -136.69 VdW: -28.31 Desolv: p=87.47, ap=3.39
58 895032 -74.13 PDB		Sigma Aldrich (Building Blocks):332135|ALDRICH NCI Plated 2007:97922 NCI Plated 2007:79553 Sigma Aldrich (Building Blocks):A5474|SIGMA Alfa-Aesar:43317 Sigma Aldrich (Building Blocks):51572|FLUKA Sigma Aldrich (Building Blocks):589667|ALDRICH Sigma Aldrich (Building Blocks):11250|ALDRICH ...	Mwt: 133.103 xLogP: -3.52 Charge: 0 RotBond: 3 # Protomers: 2 Contact: 1 ES: -102.17 VdW: -9.11 Desolv: p=38.04, ap=-0.89

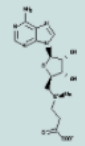
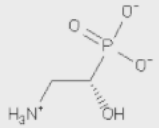
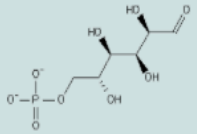
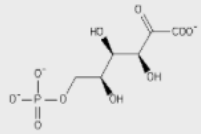
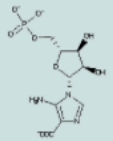
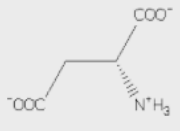
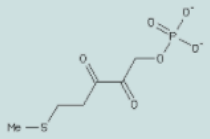
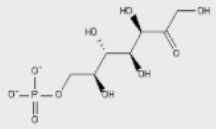
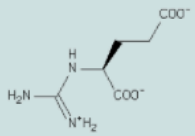
59 30725957 -73.99 PDB		Not for sale	Mwt: 139.067 xLogP: -1.21 Charge: -1 RotBond: 2 # Protomers: 1 Contact: 3 ES: -92.65 VdW: -9.42 Desolv: p=26.49, ap=1.59
60 30725295 -73.87 PDB		Not for sale	Mwt: 272.146 xLogP: -3.58 Charge: -2 RotBond: 7 # Protomers: 2 Contact: 6 ES: -93.78 VdW: -22.06 Desolv: p=37.72, ap=4.25
61 30726445 -73.43 PDB		Not for sale	Mwt: 324.245 xLogP: -4.41 Charge: -2 RotBond: 7 # Protomers: 13 Contact: 4 ES: -131.56 VdW: -21.33 Desolv: p=81.22, ap=-1.77
62 1529497 -73.41 PDB		Sigma Aldrich (Building Blocks):452432|ALDRICH Sigma Aldrich (Building Blocks):338079|ALDRICH Sigma Aldrich (Building Blocks):D1009|ALDRICH Alfa-Aesar:A10387 Sigma Aldrich (Building Blocks):659525|ALDRICH Sigma Aldrich (Building Blocks):603880|ALDRICH Sigma Aldrich (Building Blocks):44050|FLUKA NCI Plated 2007:400242 ...	Mwt: 228.288 xLogP: 3.39 Charge: -2 RotBond: 11 # Protomers: 2 Contact: 1 ES: -109.66 VdW: -15.69 Desolv: p=57.55, ap=-5.61
63 30727889 -73.38 PDB		Not for sale	Mwt: 198.091 xLogP: -2.65 Charge: -1 RotBond: 5 # Protomers: 2 Contact: 5 ES: -94.57 VdW: -14.33 Desolv: p=31.52, ap=4.00
64 901878 -73.31 PDB		Princeton BioMolecular Research:OSSL_298282 Toronto Research Chemicals:H828525 Scientific Exchange (make on demand):F-393884 FineTech:FT-0625518 eMolecules:749478 Ambinter Natural Products:Ambmdy00501000 Anward:ANW-36077 Molport:MolPort-003-665-668 ...	Mwt: 148.094 xLogP: -4.36 Charge: -1 RotBond: 3 # Protomers: 2 Contact: 2 ES: -102.97 VdW: -10.24 Desolv: p=39.45, ap=0.45
65 1531036 -73.31 PDB		Sigma Aldrich (Building Blocks):456594|ALDRICH Sigma Aldrich (Building Blocks):246379|ALDRICH Alfa-Aesar:36308 Sigma Aldrich (Building Blocks):691763|ALDRICH Alfa-Aesar:42587 Alfa-Aesar:A17271 NCI Plated 2007:19493 BioSynth:A-9800 ...	Mwt: 186.207 xLogP: 1.87 Charge: -2 RotBond: 8 # Protomers: 2 Contact: 1 ES: -109.68 VdW: -18.26 Desolv: p=58.84, ap=-4.22
66 13521326 -72.94 PDB		Not for sale	Mwt: 275.094 xLogP: -2.89 Charge: -2 RotBond: 7 # Protomers: 2 Contact: 2 ES: -116.10 VdW: -12.36 Desolv: p=55.00, ap=0.52
67 15265484 -72.76 PDB		Not for sale	Mwt: 230.162 xLogP: -4.20 Charge: -1 RotBond: 5 # Protomers: 6 Contact: 2 ES: -93.68 VdW: -18.23 Desolv: p=37.08, ap=2.06
68 30726454 -72.40 PDB		Not for sale	Mwt: 323.237 xLogP: -4.41 Charge: -3 RotBond: 7 # Protomers: 13 Contact: 4 ES: -129.79 VdW: -20.90 Desolv: p=80.04, ap=-1.75

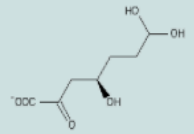
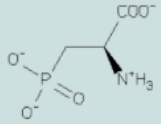
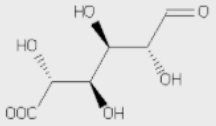
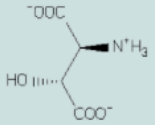
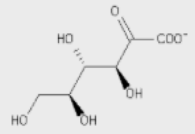
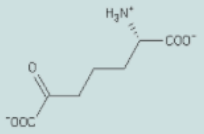
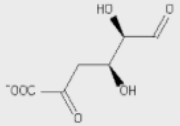
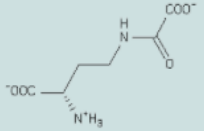
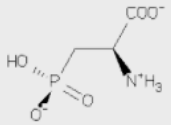
69 ☐ 4654799 -72.07 PDB		Not for sale	Mwt: 531.446 xLogP: 0.72 Charge: -1 RotBond: 9 # Protomers: 1 Contact: 6 ES: -75.52 VdW: -41.48 Desolv: p=40.42, ap=4.52
70 ☐ 895124 -72.05 PDB		Sigma Aldrich (Building Blocks):486574|ALDRICH Sigma Aldrich (Building Blocks):749435|ALDRICH Sigma Aldrich (Building Blocks):587664|ALDRICH Sigma Aldrich (Building Blocks):49480|FLUKA Vitas-M:STK017280 Alfa-Aesar:A14191 Alfa-Aesar:A17719 Labotest:LT03328670 ...	Mwt: 146.122 xLogP: -3.25 Charge: -1 RotBond: 4 # Protomers: 1 Contact: 1 ES: -110.27 VdW: -11.76 Desolv: p=51.26, ap=-1.27
71 ☐ 901781 -72.02 PDB		NCI Plated 2007:45365 Sigma Aldrich (Building Blocks):M6126|SIGMA Princeton BioMolecular Research:OSSL_297264 Scientific Exchange (make on demand):F-137682 eMolecules:592092 Labotest:LT00772127 Molport:MolPort-003-958-793 Capot Chemical:10166 ...	Mwt: 146.122 xLogP: -3.04 Charge: -1 RotBond: 3 # Protomers: 1 Contact: 1 ES: -103.87 VdW: -9.09 Desolv: p=39.69, ap=1.25
72 ☐ 30729405 -71.92 PDB		Not for sale	Mwt: 182.068 xLogP: -2.09 Charge: -2 RotBond: 4 # Protomers: 2 Contact: 3 ES: -91.23 VdW: -14.81 Desolv: p=32.79, ap=1.34
73 ☐ 49538598 -71.85 PDB		Sigma Aldrich (Building Blocks):A4910|SIGMA ZereneX Building Blocks:ZBioX-0490 Fluorochem:M01258 IBScreen Bioactives:Bio-0618 Molport BB:MolPort-001-759-594 Acros Organics:32899 Apollo Scientific:OR10671 Ryan Scientific BB:OR10671 ...	Mwt: 168.065 xLogP: -4.26 Charge: -1 RotBond: 3 # Protomers: 1 Contact: 2 ES: -96.84 VdW: -9.33 Desolv: p=32.77, ap=1.54
74 ☐ 21983366 -71.72 PDB		Sigma Aldrich (Building Blocks):467375|ALDRICH Sigma Aldrich (Building Blocks):K6250|SIGMA FineTech:FT-0624449 ChemMol:30101775 Sequoia Research Products:3470-37-9 APIChem:AC-11922 Tetrahedron Building Blocks:7465 BioSynth:K-2020 ...	Mwt: 193.131 xLogP: -3.62 Charge: -1 RotBond: 5 # Protomers: 2 Contact: 5 ES: -90.22 VdW: -15.89 Desolv: p=30.98, ap=3.40
75 ☐ 3873029 -71.62 PDB		Apollo Scientific:OR10671 IBScreen Bioactives:Bio-0618 Acros Organics:32899	Mwt: 167.057 xLogP: -4.26 Charge: -2 RotBond: 3 # Protomers: 2 Contact: 1 ES: -128.89 VdW: -9.24 Desolv: p=65.81, ap=0.70
76 ☐ 896091 -71.55 PDB		Toronto Research Chemicals:M311575 Sigma Aldrich (Building Blocks):G137|SIGMA FineTech:FT-0604674 Princeton BioMolecular BuildingBlocks:PBMR052902 American Custom Chemicals Corp.:AAA0000080	Mwt: 160.149 xLogP: -2.77 Charge: -1 RotBond: 4 # Protomers: 1 Contact: 1 ES: -100.29 VdW: -10.08 Desolv: p=39.94, ap=-1.11
77 ☐ 4654798 -71.47 PDB		Not for sale	Mwt: 210.141 xLogP: -1.55 Charge: -2 RotBond: 6 # Protomers: 1 Contact: 1 ES: -112.57 VdW: -23.85 Desolv: p=64.59, ap=0.36
78 ☐ 1530266 -71.34 PDB		Not for sale	Mwt: 242.211 xLogP: -4.77 Charge: -1 RotBond: 7 # Protomers: 1 Contact: 1 ES: -101.58 VdW: -26.44 Desolv: p=55.07, ap=1.61

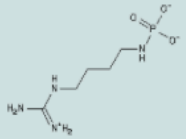
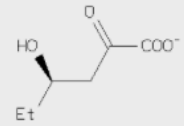
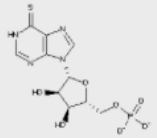
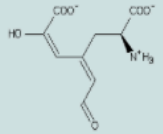
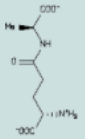
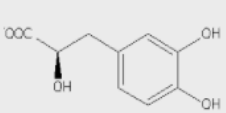
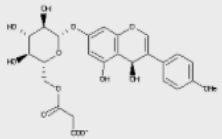
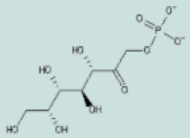
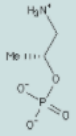
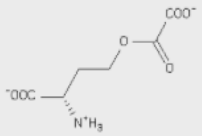
79 ☐ 1696211 -71.28 PDB		Sigma Aldrich (Building Blocks):PS306|SUPELCO Labotest:LTBB002674 TimTec Building Blocks:SBB003160 Vitas-M:STK107020 Sigma Aldrich (Building Blocks):45420|FLUKA Innovapharm BB Make on Demand:BBV-00049328 Labotest:LT00068598 TimTec Make-on-Demand:ST51046933 ...	Mwt: 248.085 xLogP: 3.07 Charge: -1 RotBond: 5 # Protomers: 1 Contact: 2 ES: -76.84 VdW: -21.57 Desolv: p=31.64, ap=-4.51	84 ☐ 4097119 -70.99 PDB		Not for sale	Mwt: 393.265 xLogP: -1.17 Charge: -1 RotBond: 8 # Protomers: 1 Contact: 5 ES: -80.20 VdW: -29.30 Desolv: p=34.81, ap=3.70
80 ☐ 4096038 -71.27 PDB		Not for sale	Mwt: 195.071 xLogP: -3.40 Charge: -2 RotBond: 5 # Protomers: 1 Contact: 1 ES: -135.44 VdW: -12.93 Desolv: p=74.69, ap=2.40	85 ☐ 22055353 -70.83 PDB		Sigma Aldrich (Building Blocks):C2196|SIGMA Toronto Research Chemicals:C997200 American Custom Chemicals Corp.:AAA0006054	Mwt: 200.217 xLogP: -4.90 Charge: -1 RotBond: 4 # Protomers: 2 Contact: 1 ES: -92.45 VdW: -12.38 Desolv: p=32.70, ap=1.30
81 ☐ 56871001 -71.19 PDB		Not for sale	Mwt: 183.076 xLogP: -1.83 Charge: -1 RotBond: 5 # Protomers: 2 Contact: 2 ES: -93.20 VdW: -13.55 Desolv: p=35.39, ap=0.18	86 ☐ 30320725 -70.83 PDB		Not for sale	Mwt: 289.153 xLogP: -4.46 Charge: -1 RotBond: 8 # Protomers: 2 Contact: 7 ES: -101.82 VdW: -20.58 Desolv: p=46.16, ap=5.41
82 ☐ 30730852 -71.08 PDB		Not for sale	Mwt: 431.342 xLogP: -0.76 Charge: -2 RotBond: 9 # Protomers: 2 Contact: 5 ES: -77.43 VdW: -29.98 Desolv: p=34.91, ap=1.42	87 ☐ 895793 -70.70 PDB		PepTech:BL802-1 NCI Plated 2007:341646 Sigma Aldrich (Building Blocks):03688|FLUKA Sigma Aldrich (Building Blocks):G1763|SIGMA Fluorochem:045390 Chembo Pharma:KB-70424 Tractus:RT-011491 Zylexa Pharma BB:ZP-BB-PT000283 ...	Mwt: 146.122 xLogP: -1.79 Charge: -1 RotBond: 4 # Protomers: 1 Contact: 1 ES: -112.42 VdW: -4.76 Desolv: p=45.00, ap=1.48
83 ☐ 3869282 -70.99 PDB		Sigma Aldrich (Building Blocks):C4418|SIGMA Princeton BioMolecular Research:OSSL_298271 Pharmeks:PHAR040154 Sigma Aldrich (Building Blocks):270881|ALDRICH Labotest:LT00452943 Ambinter Natural Products:GPL000369 eMolecules:536121 TimTec Make-on-Demand:ST51006991 ...	Mwt: 152.151 xLogP: -4.87 Charge: -1 RotBond: 3 # Protomers: 1 Contact: 1 ES: -108.79 VdW: -6.86 Desolv: p=43.11, ap=1.55	88 ☐ 1529861 -70.63 PDB		Not for sale	Mwt: 154.082 xLogP: -1.82 Charge: -1 RotBond: 3 # Protomers: 1 Contact: 1 ES: -122.56 VdW: -7.35 Desolv: p=57.03, ap=2.24

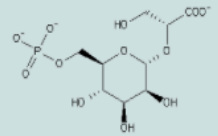
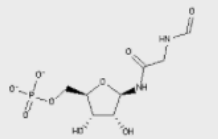
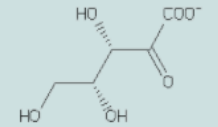
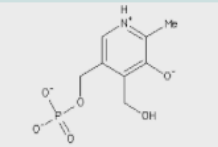
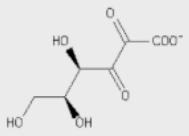
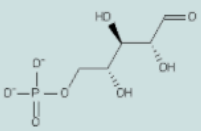
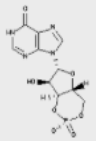
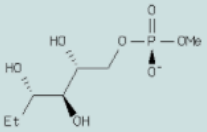
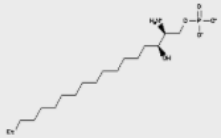
89 2123545 -70.56 PDB		Alfa-Aesar:A18601 eMolecules:31661134 Sigma Aldrich (Building Blocks):U6375|SIGMA Sigma Aldrich (Building Blocks):662666|ALDRICH Ambinter Natural Products:GPL000211 FineTech:FT-0621292 Bosche Scientific:U6624 AK Scientific:U490 ...	Mwt: 322.166 xLogP: -2.76 Charge: -2 RotBond: 4 # Protomers: 2 Contact: 5 ES: -94.57 VdW: -18.81 Desolv: p=39.28, ap=3.54
90 16343343 -70.56 PDB		Not for sale	Mwt: 291.411 xLogP: 4.80 Charge: -1 RotBond: 13 # Protomers: 1 Contact: 1 ES: -75.38 VdW: -23.19 Desolv: p=33.71, ap=-5.70
91 30724509 -70.51 PDB		Not for sale	Mwt: 190.107 xLogP: -4.02 Charge: -2 RotBond: 5 # Protomers: 5 Contact: 4 ES: -86.48 VdW: -16.39 Desolv: p=31.00, ap=1.36
92 19943047 -70.28 PDB		Sigma Aldrich (Building Blocks):A8054|SIGMA Sigma Aldrich (Building Blocks):283967|ALDRICH Sigma Aldrich (Building Blocks):A6553|SIGMA Sigma Aldrich (Building Blocks):09248|FLUKA Fluorochem:M01267 Pharmeks:PHAR177799 Sigma Aldrich (Building Blocks):A5282|SIGMA Molport:MolPort-000-838-070 ...	Mwt: 196.119 xLogP: -3.75 Charge: -1 RotBond: 5 # Protomers: 1 Contact: 2 ES: -90.14 VdW: -15.74 Desolv: p=34.59, ap=1.02
93 13542258 -70.21 PDB		Not for sale	Mwt: 388.253 xLogP: -1.16 Charge: -1 RotBond: 6 # Protomers: 3 Contact: 2 ES: -69.29 VdW: -31.60 Desolv: p=30.38, ap=0.30
94 30730847 -70.17 PDB		Not for sale	Mwt: 431.342 xLogP: -0.76 Charge: -2 RotBond: 9 # Protomers: 2 Contact: 5 ES: -98.30 VdW: -14.12 Desolv: p=40.68, ap=1.57
95 30729357 -70.12 PDB		Not for sale	Mwt: 318.282 xLogP: -5.20 Charge: -2 RotBond: 10 # Protomers: 2 Contact: 4 ES: -125.27 VdW: -18.39 Desolv: p=72.71, ap=0.84
96 4096869 -70.11 PDB		Labotest:LT00249171	Mwt: 140.055 xLogP: -2.80 Charge: -1 RotBond: 2 # Protomers: 1 Contact: 2 ES: -122.04 VdW: -6.58 Desolv: p=55.21, ap=3.31
97 56871035 -70.08 PDB		Not for sale	Mwt: 185.092 xLogP: -1.65 Charge: -1 RotBond: 5 # Protomers: 2 Contact: 3 ES: -94.95 VdW: -9.20 Desolv: p=31.95, ap=2.12
98 30320708 -70.04 PDB		Sigma Aldrich (Building Blocks):X3750|SIGMA	Mwt: 229.101 xLogP: -3.74 Charge: -1 RotBond: 6 # Protomers: 2 Contact: 5 ES: -105.49 VdW: -10.64 Desolv: p=41.91, ap=4.19

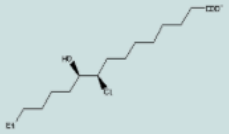
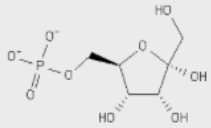
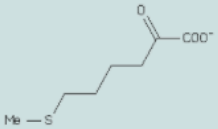
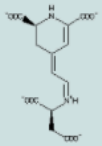
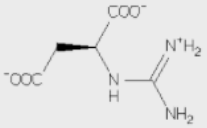
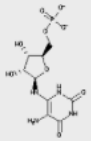
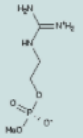
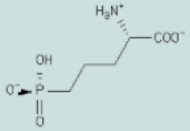
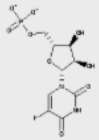
99 4096035 -69.89 PDB		Not for sale	Mwt: 245.153 xLogP: -3.57 Charge: -2 RotBond: 6 # Protomers: 1 Contact: 1 ES: -137.14 VdW: -17.76 Desolv: p=79.18, ap=5.82
100 19204143 -69.86 PDB		Labotest:LT00249171 TimTec Make-on-Demand:ST51000190 Enamine BB Make on Demand:BBV-38375483	Mwt: 141.063 xLogP: -2.80 Charge: 0 RotBond: 2 # Protomers: 2 Contact: 3 ES: -83.45 VdW: -7.44 Desolv: p=16.94, ap=4.08
101 40164487 -69.63 PDB		Not for sale	Mwt: 335.185 xLogP: -2.16 Charge: -3 RotBond: 6 # Protomers: 2 Contact: 5 ES: -103.75 VdW: -19.38 Desolv: p=52.17, ap=1.33
102 13540266 -69.62 PDB		Sigma Aldrich (Building Blocks):S0883|FLUKA TimTec Make-on-Demand:ST50767935 Innovapharm:STT-00280641 ChemMol:30108070 Prestwick Chemical:Prestw-520 Tetrahedron Building Blocks:30534 Labotest:LT00772281 Labotest Building Blocks:LT00772281 ...	Mwt: 396.384 xLogP: 2.09 Charge: -2 RotBond: 6 # Protomers: 5 Contact: 3 ES: -83.81 VdW: -33.70 Desolv: p=51.08, ap=-3.19
103 24492326 -69.62 PDB		Sigma Aldrich (Building Blocks):D7137|SIGMA Sigma Aldrich (Building Blocks):G5251|SIGMA Ambinter Natural Products:GPL000107 Sigma Aldrich (Building Blocks):51269|SIGMA Sigma Aldrich (Building Blocks):37442|SIGMA Tractus:RT-012356 ...	Mwt: 168.041 xLogP: -1.81 Charge: -2 RotBond: 4 # Protomers: 4 Contact: 3 ES: -97.02 VdW: -12.53 Desolv: p=38.34, ap=1.58
104 18182503 -69.50 PDB		Oakwood Chemical:047297 Sigma Aldrich (Building Blocks):P1781|ALDRICH Sigma Aldrich (Building Blocks):Y0001243|FLUKA Ark Pharm Building Blocks:AK108340 BePharm Building Blocks:B149448 ShangHai Biochempartner:BCP9000942 Apollo Scientific:OR17919 Fluorochem:047297 ...	Mwt: 311.281 xLogP: 0.78 Charge: -1 RotBond: 4 # Protomers: 2 Contact: 2 ES: -75.64 VdW: -37.54 Desolv: p=45.07, ap=-1.39
105 30730621 -69.43 PDB		Indofine:14-1803-8	Mwt: 291.411 xLogP: 5.12 Charge: -1 RotBond: 13 # Protomers: 2 Contact: 1 ES: -76.07 VdW: -21.40 Desolv: p=33.54, ap=-5.50
106 56870785 -69.31 PDB		Not for sale	Mwt: 227.129 xLogP: -0.16 Charge: -1 RotBond: 3 # Protomers: 2 Contact: 4 ES: -89.42 VdW: -16.61 Desolv: p=33.59, ap=3.14
107 895967 -69.29 PDB		Sigma Aldrich (Building Blocks):76157|SIGMA Toronto Research Chemicals:H942550 Ambinter Natural Products:GPL000209 Bachem:F-3335 American Custom Chemicals Corp.:CCH0007792	Mwt: 162.121 xLogP: -2.19 Charge: -1 RotBond: 4 # Protomers: 1 Contact: 2 ES: -119.04 VdW: -4.90 Desolv: p=52.01, ap=2.63
108 24720720 -69.14 PDB		Not for sale	Mwt: 139.067 xLogP: -1.21 Charge: -1 RotBond: 2 # Protomers: 1 Contact: 3 ES: -93.29 VdW: -8.16 Desolv: p=30.36, ap=1.95

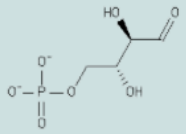
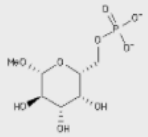
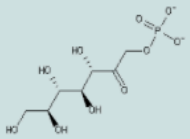
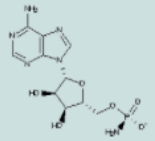
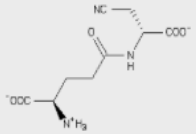
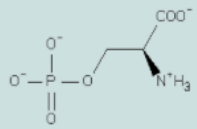
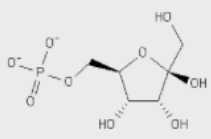
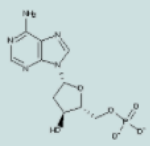
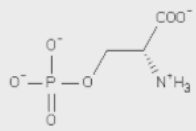
109 4096409 -69.07 PDB		Not for sale	Mwt: 397.413 xLogP: -4.00 Charge: -1 RotBond: 7 # Protomers: 3 Contact: 5 ES: -86.23 VdW: -24.56 Desolv: p=38.30, ap=3.42
110 4096868 -69.07 PDB		Labotest:LT00249171	Mwt: 140.055 xLogP: -2.80 Charge: -1 RotBond: 2 # Protomers: 1 Contact: 2 ES: -121.65 VdW: -5.76 Desolv: p=54.72, ap=3.63
111 19850142 -69.07 PDB		Indofine:BIO-188 FineTech:FT-0624563 Sequoia Research Products:54010-71-8 AK Scientific:M256 Ark Pharm Building Blocks:AK114457 BePharm Building Blocks:B132102 Tractus:TR-032428 J&K Chemical:A01975289 ...	Mwt: 258.119 xLogP: -3.31 Charge: -2 RotBond: 7 # Protomers: 2 Contact: 6 ES: -98.45 VdW: -19.87 Desolv: p=44.59, ap=4.66
112 56870848 -69.02 PDB		Not for sale	Mwt: 272.102 xLogP: -4.25 Charge: -2 RotBond: 7 # Protomers: 2 Contact: 5 ES: -106.74 VdW: -21.69 Desolv: p=57.40, ap=2.01
113 4096533 -69.01 PDB		ChemMol:44031991 Amadis Chemical:A847932	Mwt: 337.181 xLogP: -2.41 Charge: -2 RotBond: 5 # Protomers: 4 Contact: 5 ES: -132.86 VdW: -24.92 Desolv: p=83.73, ap=5.04
114 895218 -68.93 PDB		Alfa-Aesar:A13646 Sigma Aldrich (Building Blocks):488941|ALDRICH Sigma Aldrich (Building Blocks):589667|ALDRICH Sigma Aldrich (Building Blocks):11240|ALDRICH Alfa-Aesar:B21184 Labotest:LT03328272 IBScreen BuildingBlocks:BB_NC-2214 TimTec Building Blocks:SBB015070 ...	Mwt: 133.103 xLogP: -3.52 Charge: 0 RotBond: 3 # Protomers: 2 Contact: 1 ES: -103.77 VdW: -2.41 Desolv: p=38.10, ap=-0.84
115 3869224 -68.88 PDB		Not for sale	Mwt: 240.173 xLogP: -1.85 Charge: -2 RotBond: 7 # Protomers: 1 Contact: 1 ES: -138.61 VdW: -15.07 Desolv: p=84.21, ap=0.59
116 30320729 -68.85 PDB		Not for sale	Mwt: 289.153 xLogP: -4.46 Charge: -1 RotBond: 8 # Protomers: 2 Contact: 7 ES: -100.32 VdW: -16.80 Desolv: p=42.33, ap=5.92
117 1529598 -68.77 PDB		Sigma Aldrich (Building Blocks):G7788|SIGMA Cayman Chemical:80340 Anward:ANW-36349 Tractus:TR-023852 American Custom Chemicals Corp.:NUC0003022 TCI:G0265 Molport BB:MolPort-009-018-784 ...	Mwt: 188.163 xLogP: -3.26 Charge: -1 RotBond: 6 # Protomers: 2 Contact: 1 ES: -95.90 VdW: -12.95 Desolv: p=41.61, ap=-1.54
118 16343346 -68.76 PDB		Indofine:10-1803-27-7 American Custom Chemicals Corp.:LIP0000449	Mwt: 277.428 xLogP: 6.60 Charge: -1 RotBond: 13 # Protomers: 1 Contact: 1 ES: -72.59 VdW: -22.05 Desolv: p=32.34, ap=-6.46

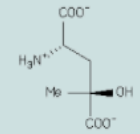
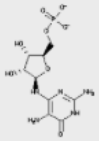
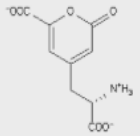
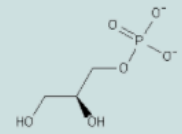
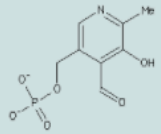
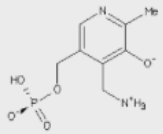
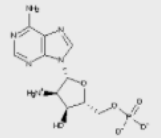
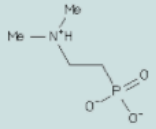
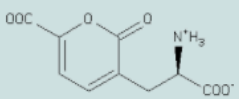
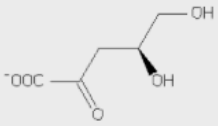
119 1530375 -68.62 PDB		Not for sale	Mwt: 191.159 xLogP: -2.40 Charge: -1 RotBond: 6 # Protomers: 1 Contact: 4 ES: -88.56 VdW: -18.44 Desolv: p=34.72, ap=3.65
120 1529380 -68.48 PDB		NCI Plated 2007:65617	Mwt: 185.243 xLogP: 2.95 Charge: -1 RotBond: 9 # Protomers: 1 Contact: 1 ES: -90.04 VdW: -12.95 Desolv: p=35.48, ap=-0.97
121 3873028 -68.46 PDB		Apollo Scientific:OR10671 NCI Plated 2007:30078 IBScreen Bioactives:Bio-0618	Mwt: 168.065 xLogP: -4.26 Charge: -1 RotBond: 3 # Protomers: 2 Contact: 1 ES: -129.59 VdW: -6.98 Desolv: p=67.40, ap=0.71
122 11616411 -68.46 PDB		NCI Plated 2007:52903 BioSynth:K-2030 Labotest:LT03328718	Mwt: 193.131 xLogP: -2.76 Charge: -1 RotBond: 5 # Protomers: 1 Contact: 5 ES: -91.81 VdW: -14.90 Desolv: p=34.22, ap=4.02
123 901877 -68.44 PDB		Princeton BioMolecular Research:OSSL_298282 eMolecules:749478 Ambinter Natural Products:Ambmdy00501000 Anward:ANW-36077 Molport:MolPort-003-665-668 Scientific Exchange (make on demand):F-393884 Tractus:RT-001415 TCI:H0947 ...	Mwt: 147.086 xLogP: -4.36 Charge: -2 RotBond: 3 # Protomers: 2 Contact: 2 ES: -93.04 VdW: -12.60 Desolv: p=36.76, ap=0.45
124 25669369 -68.38 PDB		FineTech:FT-0612737 Acros Organics:34542 American Custom Chemicals Corp.:CCH0012490	Mwt: 193.131 xLogP: -3.62 Charge: -1 RotBond: 5 # Protomers: 1 Contact: 5 ES: -89.73 VdW: -11.84 Desolv: p=30.51, ap=2.67
125 1529771 -68.31 PDB		Not for sale	Mwt: 188.159 xLogP: -3.57 Charge: -1 RotBond: 6 # Protomers: 1 Contact: 1 ES: -106.05 VdW: -14.76 Desolv: p=51.04, ap=1.46
126 1529844 -68.26 PDB		Not for sale	Mwt: 175.116 xLogP: -2.47 Charge: -1 RotBond: 5 # Protomers: 1 Contact: 3 ES: -89.11 VdW: -15.40 Desolv: p=35.10, ap=1.14
127 1531039 -68.26 PDB		Not for sale	Mwt: 189.147 xLogP: -3.11 Charge: -1 RotBond: 5 # Protomers: 1 Contact: 1 ES: -105.10 VdW: -16.05 Desolv: p=50.05, ap=2.84
128 49538597 -68.24 PDB		Sigma Aldrich (Building Blocks):A154|ALDRICH ZereneX Building Blocks:ZBioX-0490 Fluorochem:M01259 IBScreen Bioactives:Bio-0618 Sigma Aldrich (Building Blocks):A4910|SIGMA Molport BB:MolPort-001-759-594 Acros Organics:32899 Apollo Scientific:OR10671 ...	Mwt: 168.065 xLogP: -4.26 Charge: -1 RotBond: 3 # Protomers: 1 Contact: 2 ES: -84.12 VdW: -11.68 Desolv: p=25.83, ap=1.72

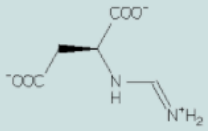
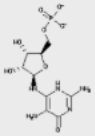
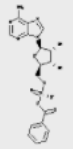
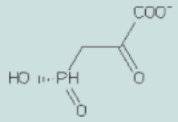
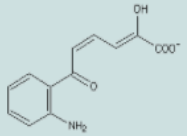
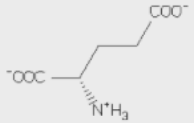
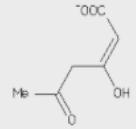
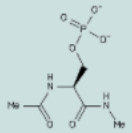
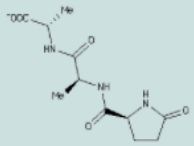
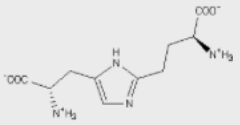
129 1529510 -68.23 PDB		Not for sale	Mwt: 209.166 xLogP: -1.78 Charge: -1 RotBond: 7 # Protomers: 1 Contact: 1 ES: -152.62 VdW: -11.10 Desolv: p=94.50, ap=0.99	134 1530552 -68.01 PDB		Not for sale	Mwt: 145.134 xLogP: -0.77 Charge: -1 RotBond: 4 # Protomers: 1 Contact: 2 ES: -89.69 VdW: -12.59 Desolv: p=33.62, ap=0.65
130 13523483 -68.22 PDB		Not for sale	Mwt: 362.26 xLogP: -2.14 Charge: -2 RotBond: 4 # Protomers: 1 Contact: 5 ES: -152.38 VdW: -21.83 Desolv: p=105.77, ap=0.23	135 13542577 -67.99 PDB		Not for sale	Mwt: 227.172 xLogP: -3.06 Charge: -2 RotBond: 6 # Protomers: 5 Contact: 2 ES: -86.00 VdW: -20.47 Desolv: p=38.67, ap=-0.18
131 2384790 -68.18 PDB		Sigma Aldrich (Building Blocks):483834|ALDRICH Ambinter Natural Products:GPL000379 Bachem:G-1905 American Custom Chemicals Corp.:AAA0004125 Combi-Blocks:QB-8417	Mwt: 217.201 xLogP: -4.24 Charge: -1 RotBond: 6 # Protomers: 1 Contact: 1 ES: -107.80 VdW: -15.52 Desolv: p=52.49, ap=2.65	136 895535 -67.98 PDB		AK Scientific:X1119 Ambinter Natural Products:GPN001280 Selleck BioChemicals NP:sodium-danshensu-S2401 ChemMol:30113666 Molport:MolPort-016-633-347 ChemMol:30113661 APIChem:AC-24422 Sigma Aldrich (Building Blocks):39363|FLUKA ...	Mwt: 197.166 xLogP: -0.25 Charge: -1 RotBond: 3 # Protomers: 1 Contact: 4 ES: -83.48 VdW: -19.87 Desolv: p=32.31, ap=3.07
132 4096156 -68.10 PDB		Not for sale	Mwt: 533.462 xLogP: -0.11 Charge: -1 RotBond: 9 # Protomers: 1 Contact: 6 ES: -85.58 VdW: -26.01 Desolv: p=37.00, ap=6.49	137 56871231 -67.90 PDB		Not for sale	Mwt: 289.153 xLogP: -4.46 Charge: -1 RotBond: 8 # Protomers: 2 Contact: 7 ES: -86.36 VdW: -21.14 Desolv: p=33.46, ap=6.13
133 1529860 -68.02 PDB		Not for sale	Mwt: 154.082 xLogP: -1.82 Charge: -1 RotBond: 3 # Protomers: 1 Contact: 1 ES: -118.09 VdW: -7.86 Desolv: p=55.77, ap=2.17	138 13352818 -67.88 PDB		Not for sale	Mwt: 190.131 xLogP: -3.66 Charge: -1 RotBond: 6 # Protomers: 1 Contact: 1 ES: -101.02 VdW: -14.73 Desolv: p=48.25, ap=-0.38

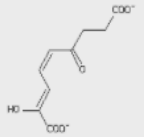
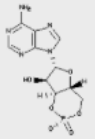
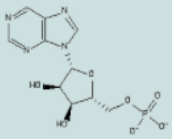
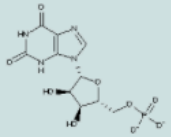
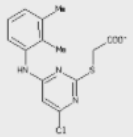
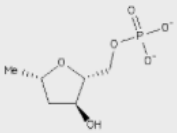
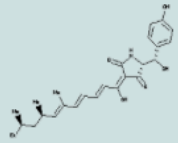
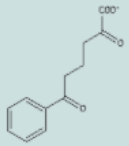
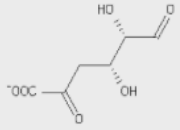
139 30731410 -67.87 PDB		Not for sale	Mwt: 346.181 xLogP: -4.09 Charge: -2 RotBond: 7 # Protomers: 2 Contact: 6 ES: -110.54 VdW: -28.05 Desolv: p=64.51, ap=6.21
140 4096399 -67.84 PDB		Not for sale	Mwt: 312.171 xLogP: -2.73 Charge: -2 RotBond: 6 # Protomers: 1 Contact: 5 ES: -123.74 VdW: -30.00 Desolv: p=79.70, ap=6.21
141 30724564 -67.84 PDB		Not for sale	Mwt: 163.105 xLogP: -3.19 Charge: -1 RotBond: 4 # Protomers: 1 Contact: 4 ES: -84.90 VdW: -15.02 Desolv: p=30.16, ap=1.92
142 24609815 -67.70 PDB		Not for sale	Mwt: 248.151 xLogP: -1.21 Charge: -1 RotBond: 4 # Protomers: 4 Contact: 4 ES: -87.86 VdW: -15.99 Desolv: p=33.28, ap=2.87
143 56874437 -67.53 PDB		Not for sale	Mwt: 217.31 xLogP: 1.82 Charge: -1 RotBond: 9 # Protomers: 1 Contact: 1 ES: -72.64 VdW: -20.96 Desolv: p=30.99, ap=-4.93
144 30725382 -67.51 PDB		Not for sale	Mwt: 191.115 xLogP: -4.02 Charge: -1 RotBond: 5 # Protomers: 1 Contact: 4 ES: -86.44 VdW: -17.78 Desolv: p=34.51, ap=2.20
145 22116391 -67.47 PDB		Sigma Aldrich (Building Blocks):83875|SIGMA Sigma Aldrich (Building Blocks):292060|ALDRICH J&K Chemical:A01292927 AK Scientific:69155 Molport BB:MolPort-003-939-233 Combi-Blocks:QB-0714	Mwt: 228.093 xLogP: -2.88 Charge: -2 RotBond: 6 # Protomers: 2 Contact: 5 ES: -92.44 VdW: -18.66 Desolv: p=39.91, ap=3.72
146 4095503 -67.44 PDB		Not for sale	Mwt: 329.185 xLogP: -2.67 Charge: -1 RotBond: 1 # Protomers: 1 Contact: 2 ES: -86.59 VdW: -25.62 Desolv: p=41.28, ap=3.49
147 56870818 -67.42 PDB		Not for sale	Mwt: 243.172 xLogP: -1.55 Charge: -1 RotBond: 7 # Protomers: 1 Contact: 4 ES: -91.24 VdW: -10.40 Desolv: p=32.31, ap=1.92
148 8216815 -67.41 PDB		Not for sale	Mwt: 380.486 xLogP: 4.73 Charge: -1 RotBond: 18 # Protomers: 1 Contact: 2 ES: -135.26 VdW: -20.22 Desolv: p=85.00, ap=3.07

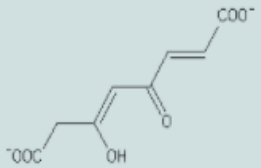
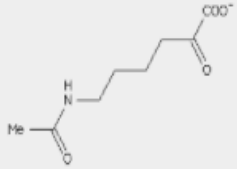
149 4654985 -67.40 PDB		Not for sale	Mwt: 305.866 xLogP: 5.53 Charge: -1 RotBond: 14 # Protomers: 1 Contact: 2 ES: -78.86 VdW: -23.07 Desolv: p=34.15, ap=0.38	154 53683550 -66.80 PDB		Not for sale	Mwt: 259.127 xLogP: -2.71 Charge: -1 RotBond: 4 # Protomers: 2 Contact: 6 ES: -97.03 VdW: -12.63 Desolv: p=37.67, ap=5.19
150 16343340 -67.30 PDB		Indofine:10-1803-23 Indofine:10-1803-24 American Custom Chemicals Corp.:LIP0000449	Mwt: 277.428 xLogP: 6.60 Charge: -1 RotBond: 13 # Protomers: 1 Contact: 1 ES: -72.34 VdW: -21.19 Desolv: p=32.28, ap=-6.06	155 56874391 -66.78 PDB		Not for sale	Mwt: 175.229 xLogP: 0.30 Charge: -1 RotBond: 6 # Protomers: 1 Contact: 1 ES: -74.18 VdW: -18.08 Desolv: p=29.27, ap=-3.79
151 30726448 -66.97 PDB		Not for sale	Mwt: 325.253 xLogP: -4.41 Charge: -1 RotBond: 7 # Protomers: 13 Contact: 4 ES: -119.54 VdW: -19.30 Desolv: p=73.75, ap=-1.87	156 4096031 -66.77 PDB		Sigma Aldrich (Building Blocks):G7379|SIGMA	Mwt: 174.136 xLogP: -3.53 Charge: -1 RotBond: 5 # Protomers: 1 Contact: 1 ES: -95.19 VdW: -15.07 Desolv: p=41.15, ap=2.35
152 4095611 -66.88 PDB		Not for sale	Mwt: 352.196 xLogP: -3.11 Charge: -2 RotBond: 5 # Protomers: 1 Contact: 5 ES: -150.05 VdW: -25.12 Desolv: p=100.48, ap=7.81	157 13520273 -66.76 PDB		Not for sale	Mwt: 197.131 xLogP: -1.85 Charge: 0 RotBond: 6 # Protomers: 1 Contact: 1 ES: -88.68 VdW: -11.64 Desolv: p=33.74, ap=-0.18
153 19632959 -66.86 PDB		IBScreen Bioactives:Bio-0871 Sigma Aldrich (Building Blocks):283967|ALDRICH Sigma Aldrich (Building Blocks):A6553|SIGMA Sigma Aldrich (Building Blocks):09248|FLUKA ZereneX Building Blocks:ZBioX-0712 Fluorochem:M01268 Pharmeks:PHAR177799 Pharmeks:PHAR031188 ...	Mwt: 196.119 xLogP: -3.75 Charge: -1 RotBond: 5 # Protomers: 1 Contact: 2 ES: -91.43 VdW: -13.03 Desolv: p=36.47, ap=1.13	158 13526798 -66.76 PDB		Not for sale	Mwt: 340.156 xLogP: -2.34 Charge: -2 RotBond: 4 # Protomers: 1 Contact: 5 ES: -149.74 VdW: -19.69 Desolv: p=99.62, ap=3.05

159 32786787 -66.69 PDB		Tractus:RT-012181	Mwt: 198.067 xLogP: -2.46 Charge: -2 RotBond: 5 # Protomers: 2 Contact: 4 ES: -91.20 VdW: -17.00 Desolv: p=38.25, ap=3.27
160 56871010 -66.68 PDB		Not for sale	Mwt: 273.154 xLogP: -2.69 Charge: -1 RotBond: 4 # Protomers: 2 Contact: 6 ES: -79.46 VdW: -22.57 Desolv: p=30.61, ap=4.74
161 56871230 -66.63 PDB		Not for sale	Mwt: 289.153 xLogP: -4.46 Charge: -1 RotBond: 8 # Protomers: 2 Contact: 7 ES: -95.61 VdW: -14.93 Desolv: p=38.39, ap=5.52
162 12496448 -66.59 PDB		Cayman Chemical:53400 Cayman Chemical:10009993 Molport BB:MolPort-009-019-268	Mwt: 313.458 xLogP: 4.81 Charge: -1 RotBond: 15 # Protomers: 1 Contact: 3 ES: -74.02 VdW: -24.78 Desolv: p=34.09, ap=-1.87
163 4096224 -66.55 PDB		Sigma Aldrich (Building Blocks):A2627|SIGMA Toronto Research Chemicals:A281800	Mwt: 345.232 xLogP: -1.82 Charge: -1 RotBond: 4 # Protomers: 3 Contact: 2 ES: -72.16 VdW: -32.88 Desolv: p=32.03, ap=6.46
164 1530269 -66.54 PDB		Not for sale	Mwt: 242.211 xLogP: -4.77 Charge: -1 RotBond: 7 # Protomers: 1 Contact: 1 ES: -106.64 VdW: -21.71 Desolv: p=60.03, ap=1.79
165 3869280 -66.53 PDB		Sigma Aldrich (Building Blocks):79710|SIGMA Sigma Aldrich (Building Blocks):P0678|SIGMA Scientific Exchange (make on demand):M-982071 TimTec Building Blocks:SBB000251 Aldrich CPR:S364827|ALDRICH Labotest:LT00451670 Ambinter Natural Products:GPL000109 FineTech:FT-0625495 ...	Mwt: 183.056 xLogP: -4.29 Charge: -2 RotBond: 4 # Protomers: 2 Contact: 1 ES: -128.55 VdW: -14.37 Desolv: p=75.40, ap=0.98
166 56874962 -66.52 PDB		Not for sale	Mwt: 259.127 xLogP: -2.71 Charge: -1 RotBond: 4 # Protomers: 2 Contact: 6 ES: -101.77 VdW: -8.37 Desolv: p=37.84, ap=5.78
167 1713574 -66.51 PDB		Labotest:LT03328564 ChemMol:44021758 Labotest:LT03328563 Sigma Aldrich (Building Blocks):852198|ALDRICH Sigma Aldrich (Building Blocks):D6250|SIGMA ChemMol:44022372 Ambinter Natural Products:GPL000237 TimTec Make-on-Demand:ST51006922 ...	Mwt: 329.209 xLogP: -1.56 Charge: -2 RotBond: 4 # Protomers: 2 Contact: 4 ES: -62.81 VdW: -29.46 Desolv: p=24.87, ap=0.89
168 3869281 -66.51 PDB		Sigma Aldrich (Building Blocks):79710|SIGMA Scientific Exchange (make on demand):M-982071 TimTec Building Blocks:SBB000251 Aldrich CPR:S364827|ALDRICH Labotest:LT00451670 eMolecules:530918 TimTec Make-on-Demand:ST51006697 Indofine:04-2317 ...	Mwt: 184.064 xLogP: -4.29 Charge: -1 RotBond: 4 # Protomers: 2 Contact: 1 ES: -124.55 VdW: -14.02 Desolv: p=71.12, ap=0.95

169 895989 -66.42 PDB		Not for sale	Mwt: 176.148 xLogP: -3.72 Charge: -1 RotBond: 4 # Protomers: 1 Contact: 2 ES: -104.72 VdW: -7.26 Desolv: p=43.16, ap=2.40	174 13526579 -66.19 PDB		Not for sale	Mwt: 351.212 xLogP: -2.95 Charge: -2 RotBond: 5 # Protomers: 2 Contact: 5 ES: -95.66 VdW: -21.40 Desolv: p=45.21, ap=5.66
170 895994 -66.42 PDB			Mwt: 226.164 xLogP: -2.72 Charge: -1 RotBond: 4 # Protomers: 1 Contact: 3 ES: -93.29 VdW: -19.15 Desolv: p=45.05, ap=0.96	175 3830895 -66.14 PDB		Sigma Aldrich (Building Blocks):50057|FLUKA Alfa-Aesar:A16269 ChemBridge:5106739 Innovapharm BB Make on Demand:BBV-00053777 Indofine:59-1220 Pharmeks:PHAR104989 Toronto Research Chemicals:G598750 Sigma Aldrich (Building Blocks):17766|SIGMA ...	Mwt: 171.065 xLogP: -2.26 Charge: -1 RotBond: 4 # Protomers: 2 Contact: 4 ES: -92.23 VdW: -11.11 Desolv: p=33.20, ap=4.00
171 1532514 -66.33 PDB		Alfa-Aesar:A12323 Sigma Aldrich (Building Blocks):82870|SIGMA Sigma Aldrich (Building Blocks):P9255|SIGMA eMolecules:1988700 Ambinter Natural Products:GPL000387 Labotest:LT00451690 TimTec Make-on-Demand:ST51006706 TimTec Building Blocks:SBB065295 ...	Mwt: 245.127 xLogP: -0.76 Charge: -2 RotBond: 4 # Protomers: 3 Contact: 4 ES: -75.75 VdW: -18.29 Desolv: p=27.08, ap=0.63	176 24412161 -66.13 PDB		Not for sale	Mwt: 247.167 xLogP: -1.37 Charge: -1 RotBond: 4 # Protomers: 6 Contact: 4 ES: -137.71 VdW: -13.33 Desolv: p=83.42, ap=1.48
172 4095693 -66.30 PDB		Not for sale	Mwt: 345.232 xLogP: -2.97 Charge: -1 RotBond: 4 # Protomers: 3 Contact: 4 ES: -131.38 VdW: -18.54 Desolv: p=77.19, ap=6.43	177 1530261 -66.04 PDB		Not for sale	Mwt: 152.11 xLogP: -0.92 Charge: -1 RotBond: 3 # Protomers: 1 Contact: 1 ES: -130.05 VdW: -4.18 Desolv: p=68.27, ap=-0.09
173 895997 -66.25 PDB		Not for sale	Mwt: 226.164 xLogP: -2.72 Charge: -1 RotBond: 4 # Protomers: 1 Contact: 2 ES: -77.68 VdW: -22.05 Desolv: p=32.56, ap=0.91	178 1532725 -66.04 PDB			Mwt: 147.106 xLogP: -2.28 Charge: -1 RotBond: 4 # Protomers: 1 Contact: 3 ES: -93.91 VdW: -12.32 Desolv: p=36.11, ap=4.08

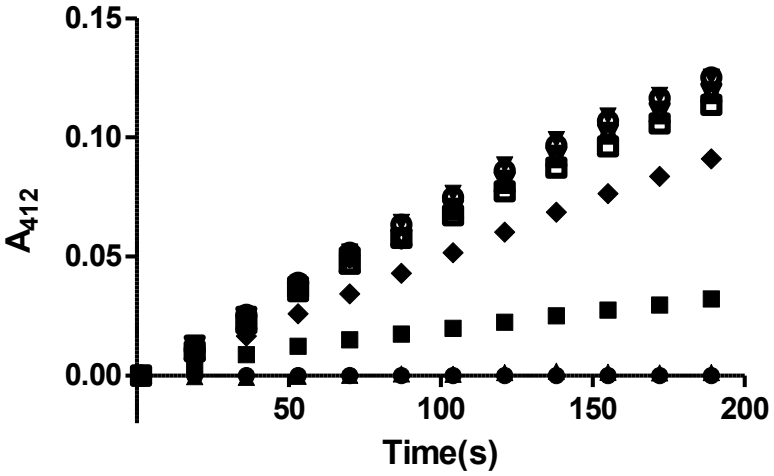
179 4096096 -66.02 PDB		Not for sale	Mwt: 159.121 xLogP: -3.00 Charge: -1 RotBond: 5 # Protomers: 1 Contact: 1 ES: -100.51 VdW: -8.79 Desolv: p=42.36, ap=0.92	184 8217107 -65.89 PDB		Not for sale	Mwt: 351.212 xLogP: -2.95 Charge: -2 RotBond: 5 # Protomers: 1 Contact: 5 ES: -120.26 VdW: -30.39 Desolv: p=79.42, ap=5.33
180 13543808 -66.02 PDB		Not for sale	Mwt: 450.324 xLogP: 0.57 Charge: -1 RotBond: 7 # Protomers: 3 Contact: 5 ES: -73.35 VdW: -25.76 Desolv: p=33.72, ap=-0.63	185 1530411 -65.87 PDB		Not for sale	Mwt: 151.034 xLogP: -3.61 Charge: -1 RotBond: 3 # Protomers: 1 Contact: 2 ES: -89.08 VdW: -14.29 Desolv: p=37.54, ap=-0.04
181 16343337 -65.92 PDB			Mwt: 232.215 xLogP: 0.85 Charge: -1 RotBond: 4 # Protomers: 1 Contact: 2 ES: -70.79 VdW: -25.42 Desolv: p=30.80, ap=-0.51	186 1482113 -65.78 PDB		Sigma Aldrich (Building Blocks):486574|ALDRICH NCI Plated 2007:88574 NCI Plated 2007:77686 Sigma Aldrich (Building Blocks):G5667|SIGMA ChemBridge:5102620 Sigma Aldrich (Building Blocks):749435|ALDRICH Sigma Aldrich (Building Blocks):607851|ALDRICH Sigma Aldrich (Building Blocks):49605|ALDRICH ...	Mwt: 146.122 xLogP: -3.25 Charge: -1 RotBond: 4 # Protomers: 1 Contact: 1 ES: -101.12 VdW: -8.07 Desolv: p=44.21, ap=-0.80
182 1529279 -65.90 PDB		Not for sale	Mwt: 143.118 xLogP: -0.44 Charge: -1 RotBond: 3 # Protomers: 1 Contact: 2 ES: -86.91 VdW: -14.80 Desolv: p=34.58, ap=1.23	187 56870853 -65.77 PDB		Not for sale	Mwt: 239.144 xLogP: -2.75 Charge: -1 RotBond: 5 # Protomers: 2 Contact: 2 ES: -85.19 VdW: -20.59 Desolv: p=38.50, ap=1.51
183 56870926 -65.90 PDB		Not for sale	Mwt: 270.265 xLogP: -3.53 Charge: -1 RotBond: 5 # Protomers: 1 Contact: 2 ES: -62.14 VdW: -29.23 Desolv: p=25.50, ap=-0.03	188 5955038 -65.76 PDB		Not for sale	Mwt: 256.262 xLogP: -5.03 Charge: 0 RotBond: 7 # Protomers: 2 Contact: 3 ES: -113.74 VdW: -15.51 Desolv: p=62.68, ap=0.82

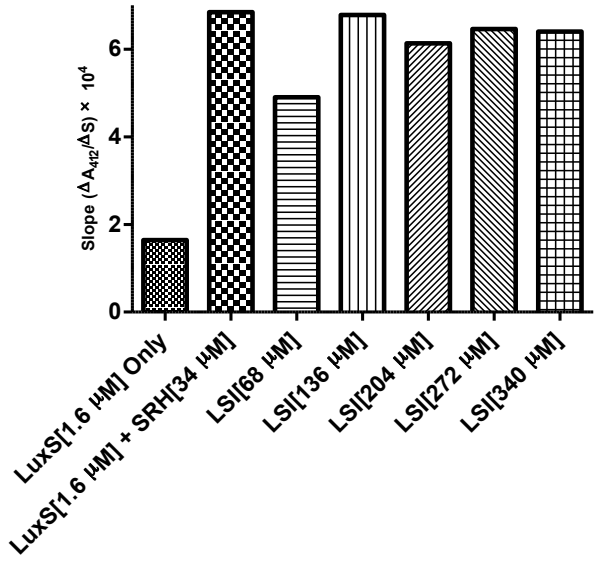
189 1532729 -65.73 PDB		Fluorochem:079457 NCI Plated 2007:90788 Sigma Aldrich (Building Blocks):T1395|SIGMA Alfa-Aesar:L04711 IBScreen BuildingBlocks:BB_NC-1525 BePharm Building Blocks:B126427 TimTec Building Blocks:SBB003484 Toronto Research Chemicals:L468725 ...	Mwt: 205.324 xLogP: 2.25 Charge: -1 RotBond: 5 # Protomers: 2 Contact: 2 ES: -80.38 VdW: -13.38 Desolv: p=32.92, ap=-4.89	194 13539883 -65.59 PDB		Not for sale	Mwt: 213.165 xLogP: -0.78 Charge: -1 RotBond: 6 # Protomers: 3 Contact: 2 ES: -96.06 VdW: -22.04 Desolv: p=54.22, ap=-1.72
190 3873977 -65.73 PDB		Prestwick Chemical:1092 Sigma Aldrich (Building Blocks):A9501|SIGMA Sigma Aldrich (Building Blocks):01894|FLUKA Sigma Aldrich (Building Blocks):A3262|SIGMA Ambinter Natural Products:GPL000358 Bosche Scientific:A6224 Oakwood Chemical:213436 Sequoia Research Products:60-92-4 ...	Mwt: 328.201 xLogP: -1.71 Charge: -1 RotBond: 1 # Protomers: 1 Contact: 3 ES: -72.50 VdW: -27.75 Desolv: p=34.21, ap=0.31	195 15614364 -65.58 PDB		Not for sale	Mwt: 330.193 xLogP: -1.93 Charge: -2 RotBond: 4 # Protomers: 1 Contact: 5 ES: -107.38 VdW: -30.21 Desolv: p=71.52, ap=0.50
191 4228292 -65.68 PDB		Not for sale	Mwt: 362.191 xLogP: -2.84 Charge: -2 RotBond: 4 # Protomers: 1 Contact: 5 ES: -147.90 VdW: -22.19 Desolv: p=98.49, ap=5.93	196 1963 -65.50 PDB		Sigma Aldrich (Building Blocks):C7081|SIGMA Cayman Chemical:70730 Fluorochem:M02679 ChemMol:97900008 Anward:ANW-31151 Molport:MolPort-003-666-579 Molport BB:MolPort-003-666-579 TCI:C1323 ...	Mwt: 322.797 xLogP: 4.67 Charge: -1 RotBond: 5 # Protomers: 1 Contact: 3 ES: -66.19 VdW: -26.20 Desolv: p=32.64, ap=-5.75
192 56870814 -65.68 PDB		Not for sale	Mwt: 211.13 xLogP: -1.16 Charge: -1 RotBond: 3 # Protomers: 2 Contact: 3 ES: -89.39 VdW: -11.83 Desolv: p=33.67, ap=1.88	197 4654682 -65.50 PDB		Not for sale	Mwt: 437.536 xLogP: 3.83 Charge: -2 RotBond: 9 # Protomers: 7 Contact: 3 ES: -54.18 VdW: -37.66 Desolv: p=26.90, ap=-0.56
193 1529739 -65.63 PDB		Not for sale	Mwt: 219.216 xLogP: 0.92 Charge: -1 RotBond: 6 # Protomers: 1 Contact: 2 ES: -78.73 VdW: -21.80 Desolv: p=35.85, ap=-0.95	198 1529843 -65.49 PDB		Not for sale	Mwt: 175.116 xLogP: -2.47 Charge: -1 RotBond: 5 # Protomers: 1 Contact: 3 ES: -88.16 VdW: -13.60 Desolv: p=34.69, ap=1.58

199 3869299 -65.45 PDB		Not for sale	Mwt: 198.13 xLogP: -1.05 Charge: -2 RotBond: 5 # Protomers: 2 Contact: 2 ES: -98.69 VdW: -18.75 Desolv: p=52.42, ap=-0.43
200 1530217 -65.44 PDB			Mwt: 186.187 xLogP: -1.08 Charge: -1 RotBond: 6 # Protomers: 1 Contact: 1 ES: -81.52 VdW: -18.44 Desolv: p=34.18, ap=0.33

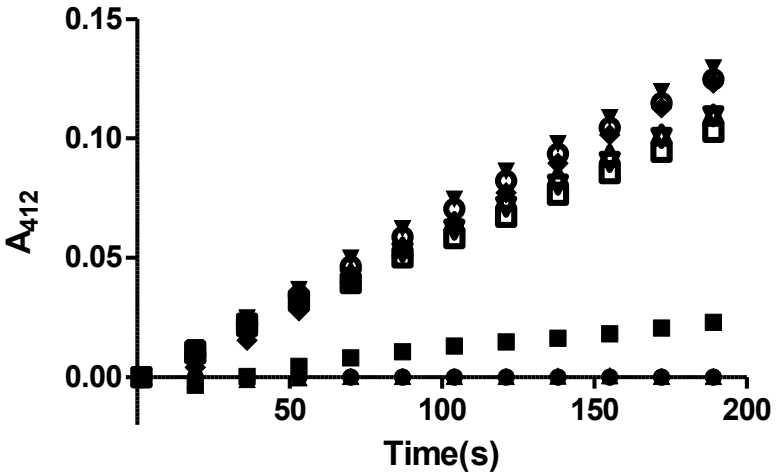
Appendix B: LuxS Inhibition Assays

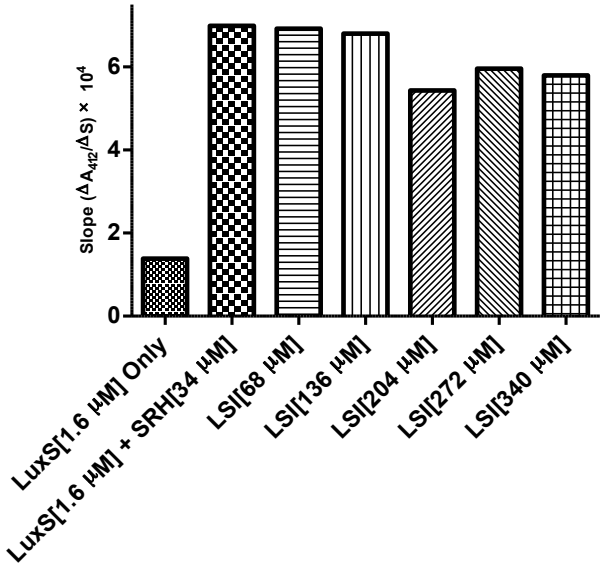
_N_-Nitro-L-arginine Trial 1





***N*-Nitro-L-arginine Trial 2**





***N*-Nitro-L-arginine Trial 3**

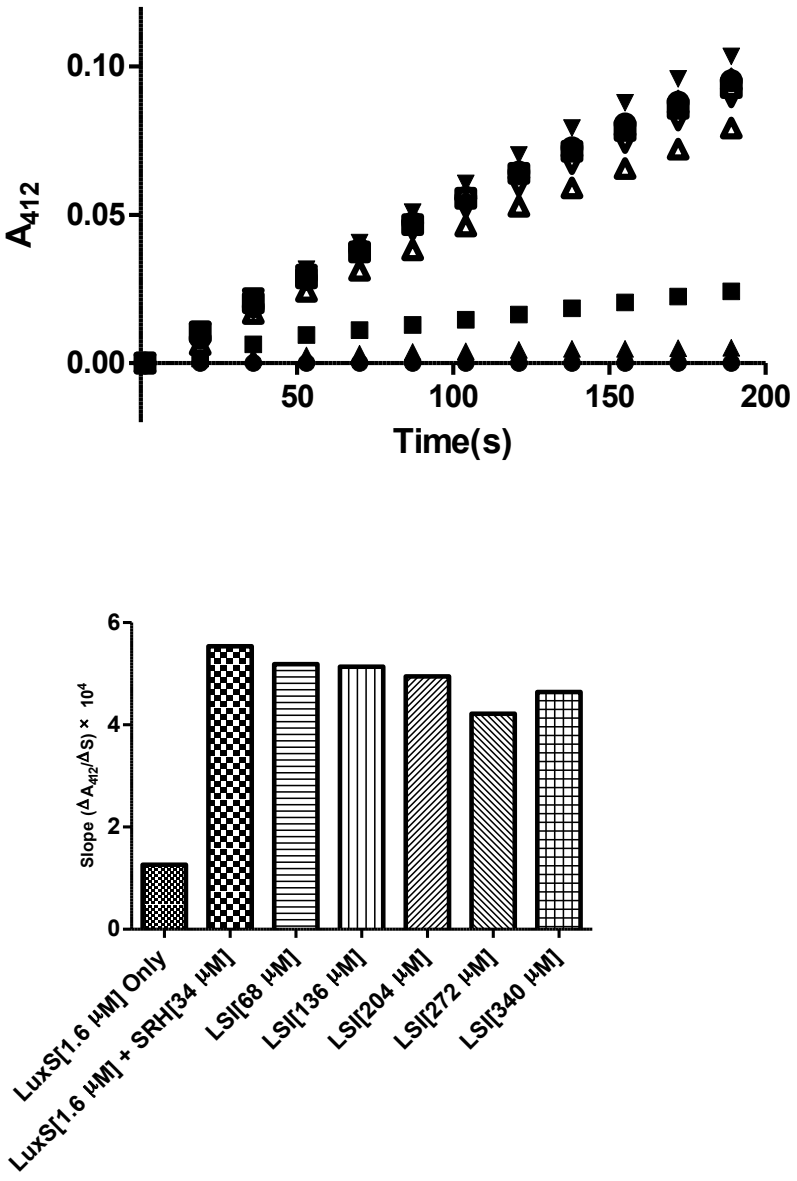
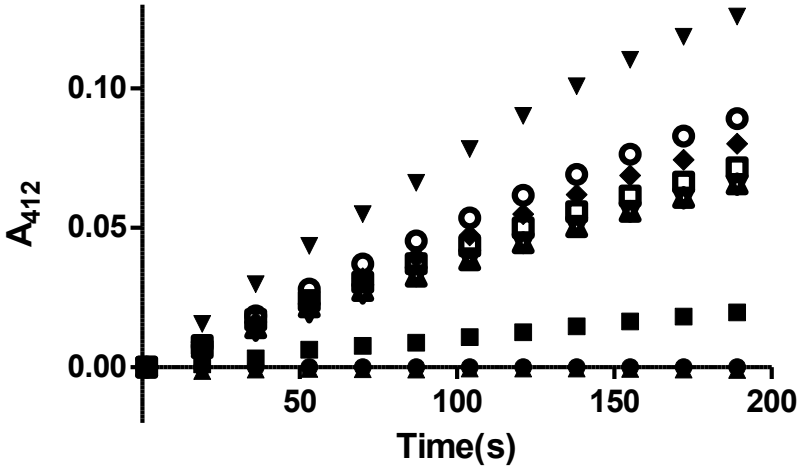
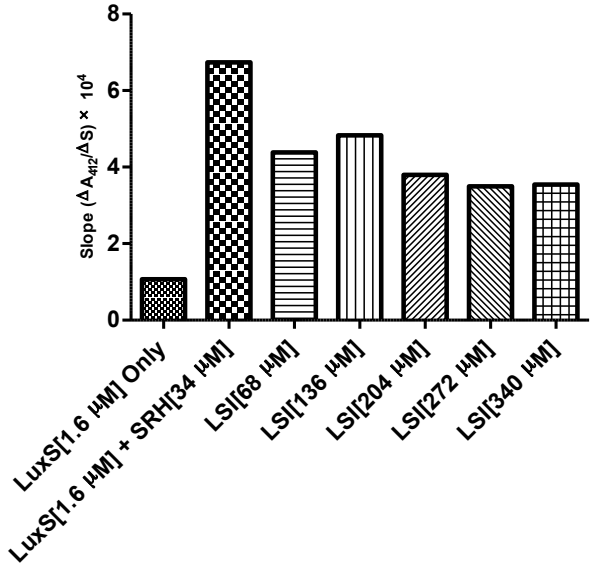


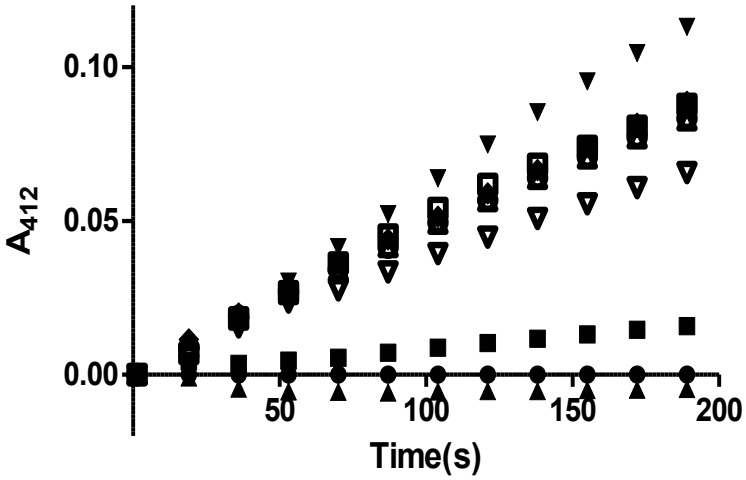
Figure 1 | *N*-Nitro-L-arginine. Graphs of all three trials for the LuxS Inhibition assay of N-nitro-L-arginine.

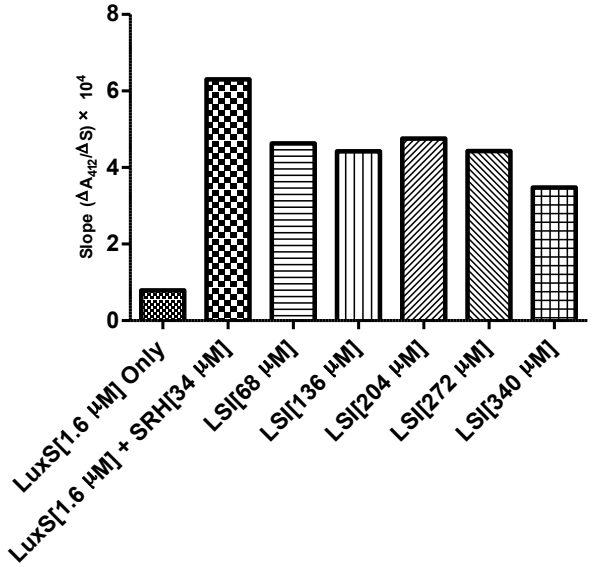
Argininosuccinate Trial 1





Argininosuccinate Trial 2





Argininosuccinate Trial 3

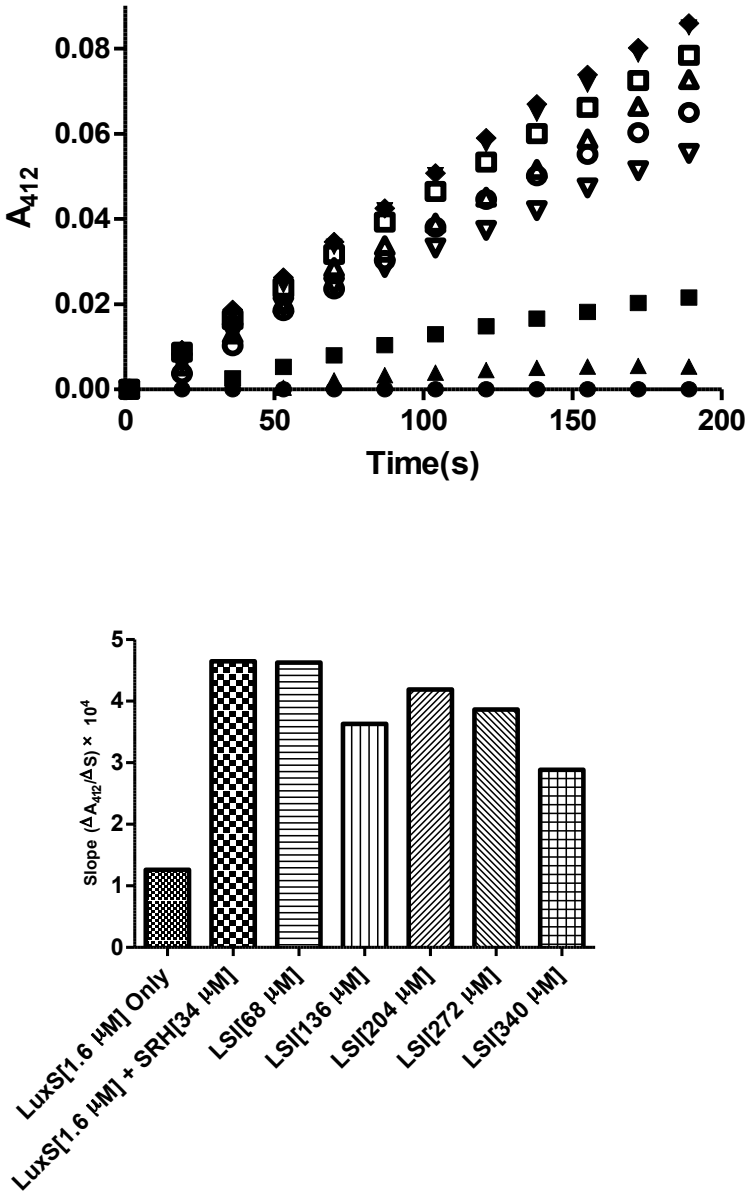
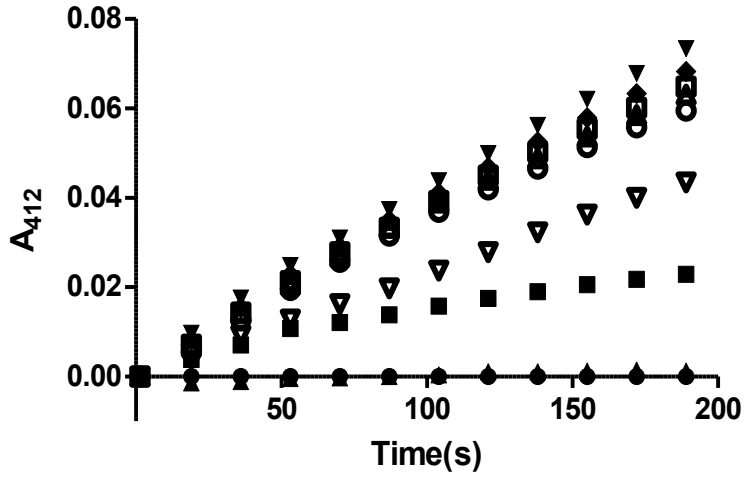
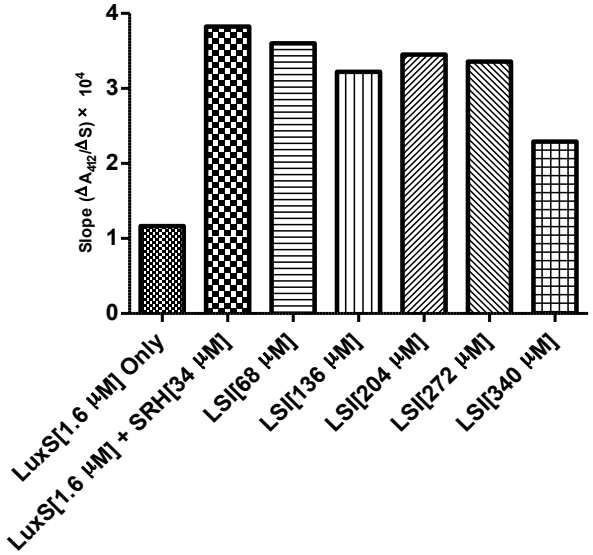


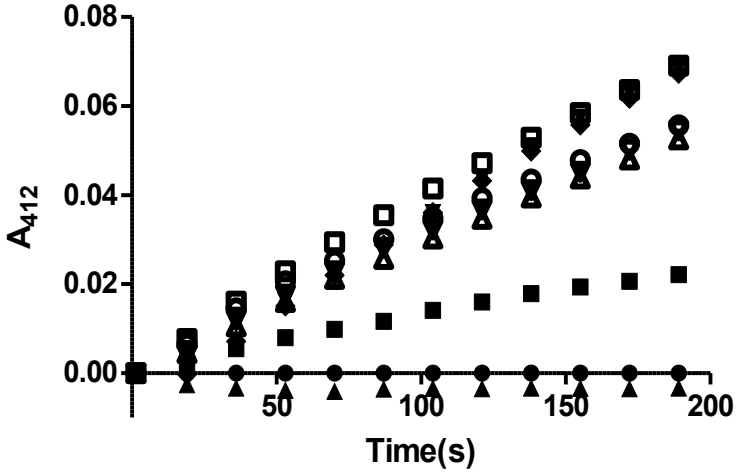
Figure 2 | Argininosuccinate. A triplicate assay of the argininosuccinate was conducted. Above shows all three individual assay.

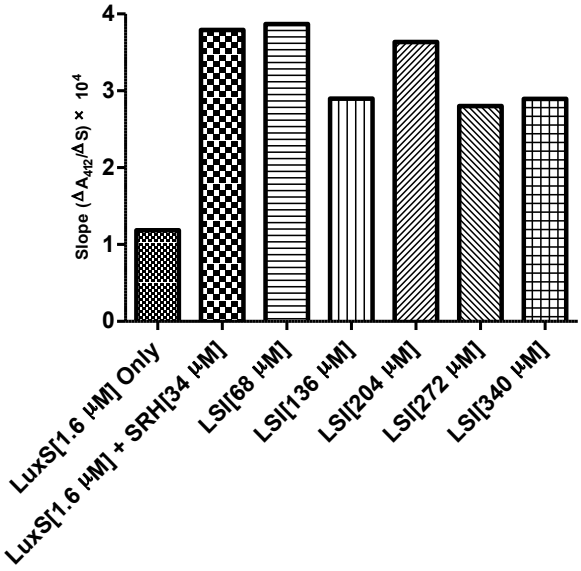
L-Arginine, HCl Trial 1





L-Arginine, HCl Trial 2





L-Arginine, HCl Trial 3

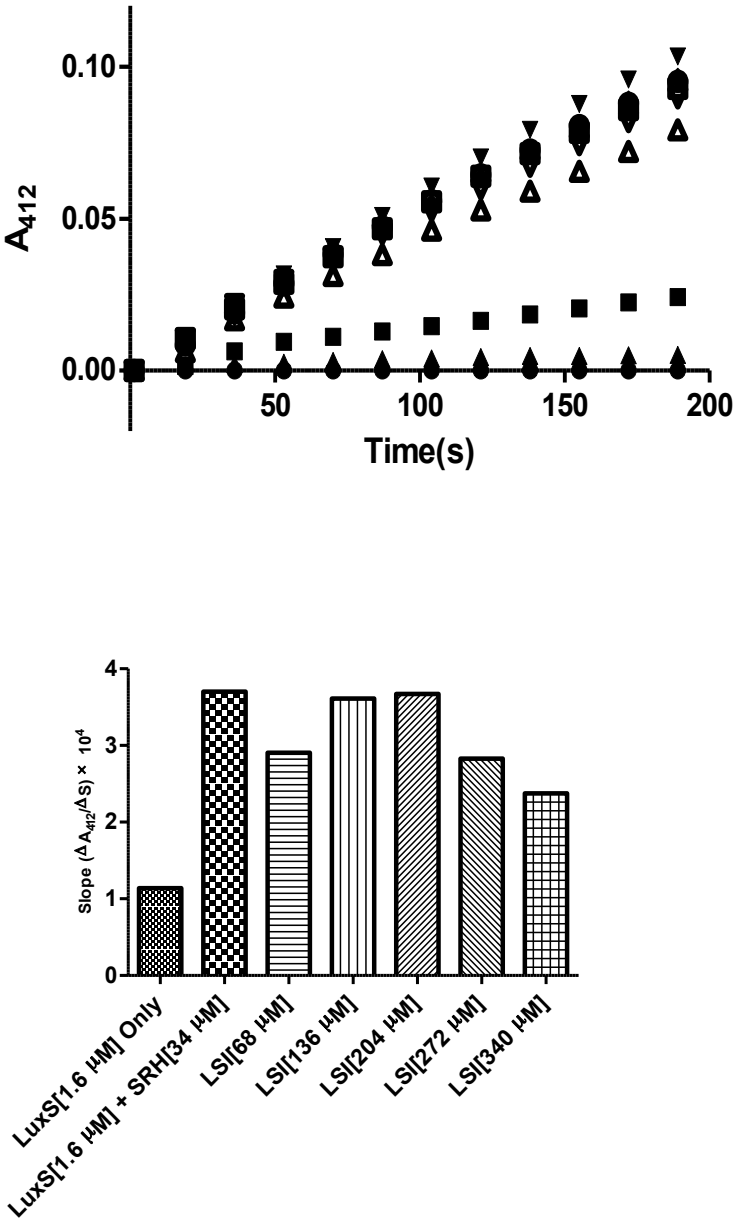
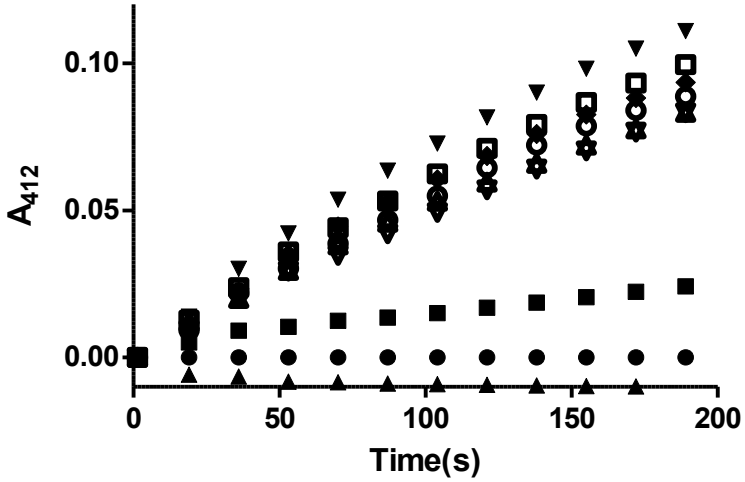
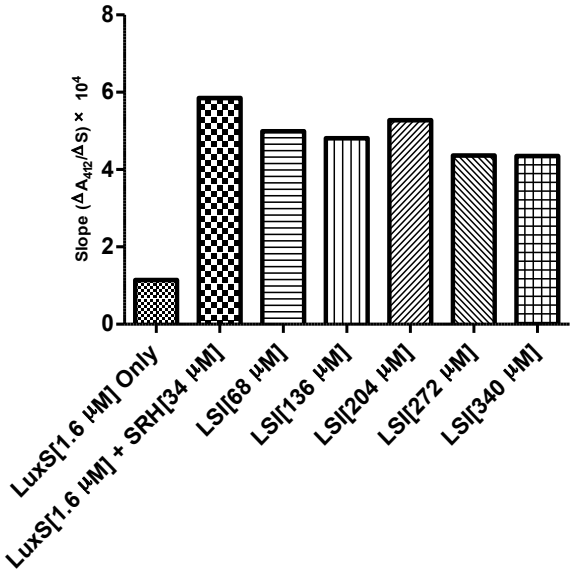


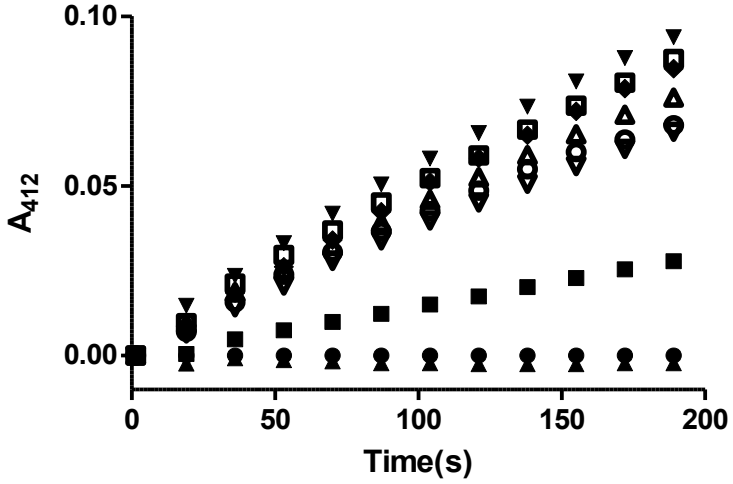
Figure 3 | L-Arginine, HCl. Graphs of all three LuxS Inhibition assay of L-Arginine, HCl.

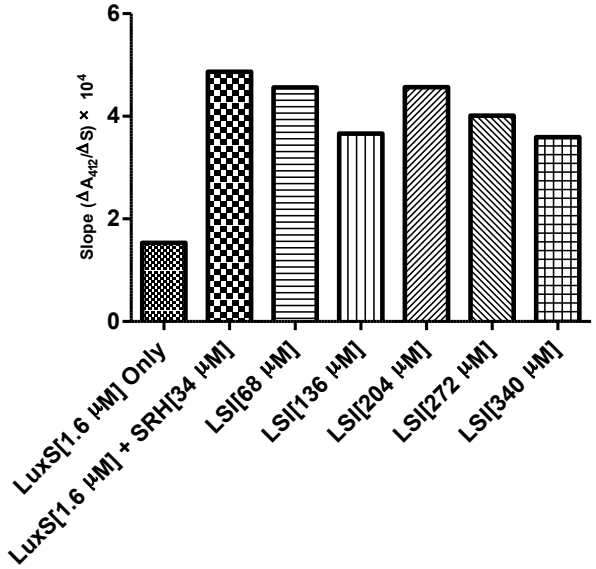
2'-Deoxyguanosine-5'-monophosphoric acid disodium salt Trial 1





2'-Deoxyguanosine-5'-monophosphoric acid disodium salt Trial 2





2'-Deoxyguanosine-5'-monophosphoric acid disodium salt Trial 3

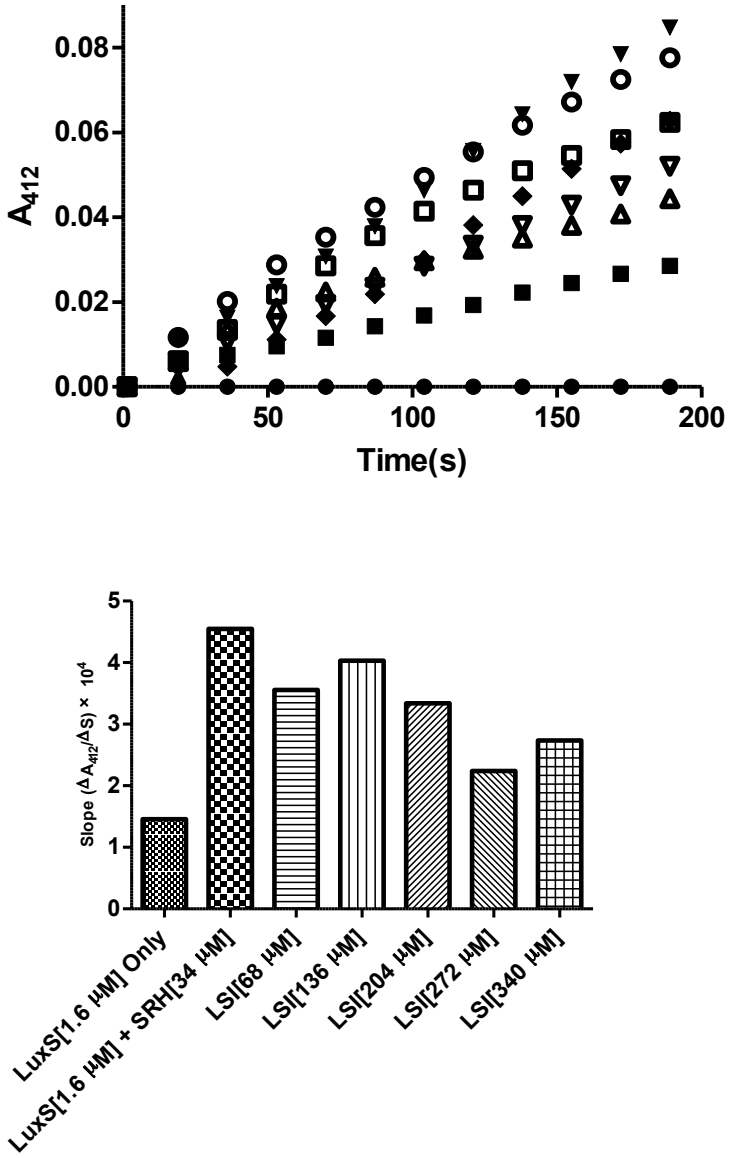
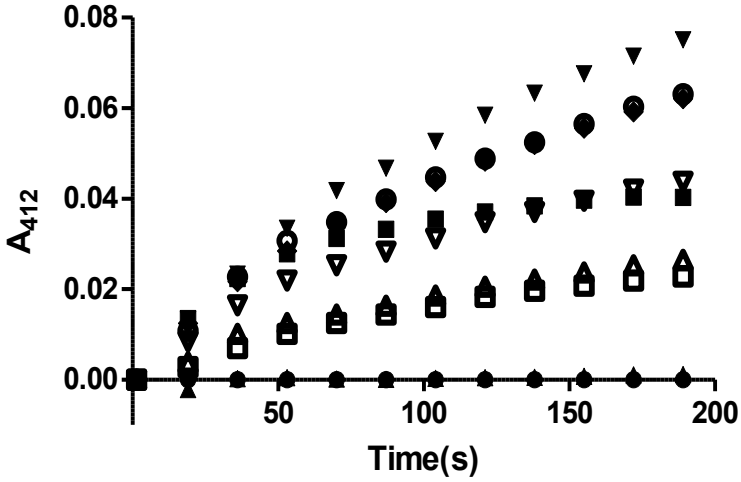
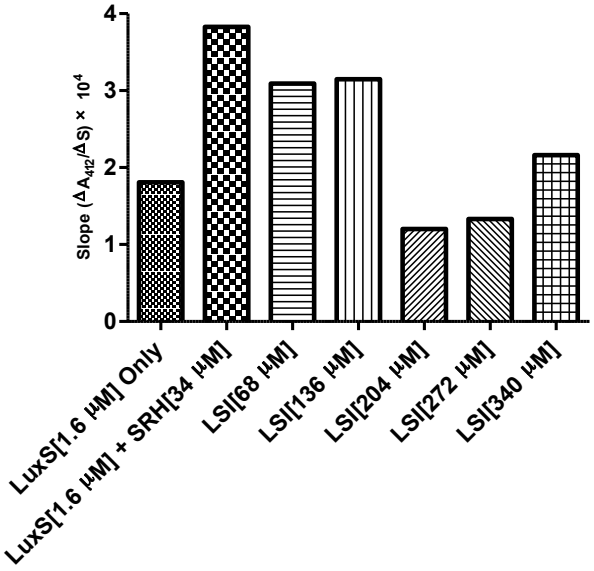


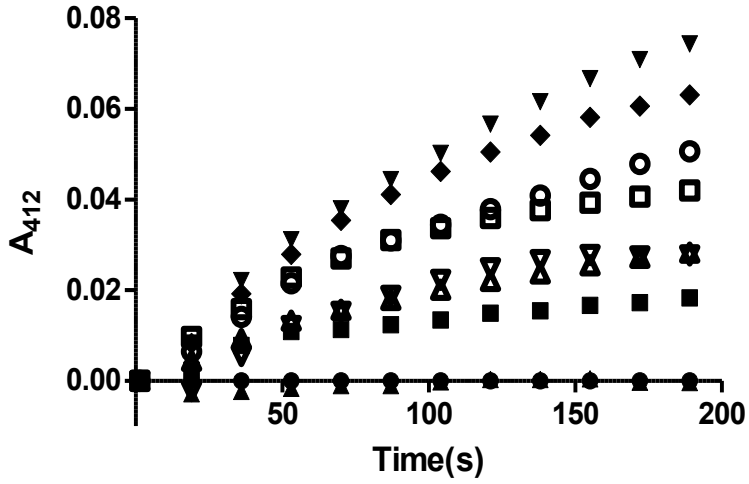
Figure 4 | 2'-Deoxyguanosine-5'-monophosphoric acid disodium salt. Graphs of all three LuxS Inhibition assay of 2'Deoxyguanosine-5'-monophosphoric acid disodium salt.

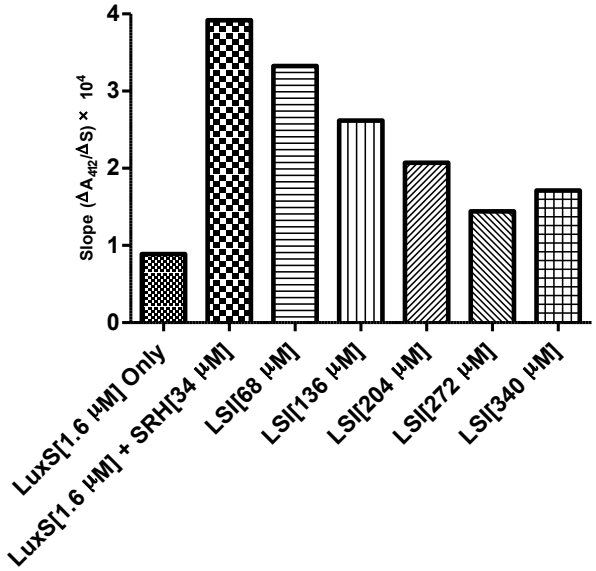
L-Lysine Trial 1



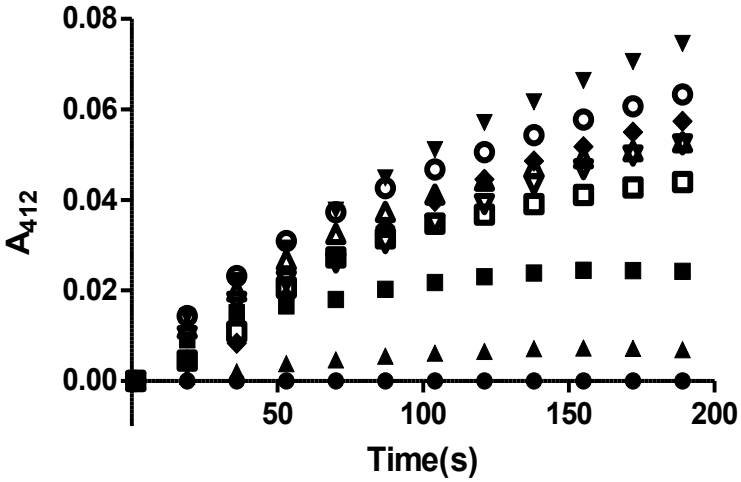


L-Lysine Trial 2





L-Lysine Trial 3



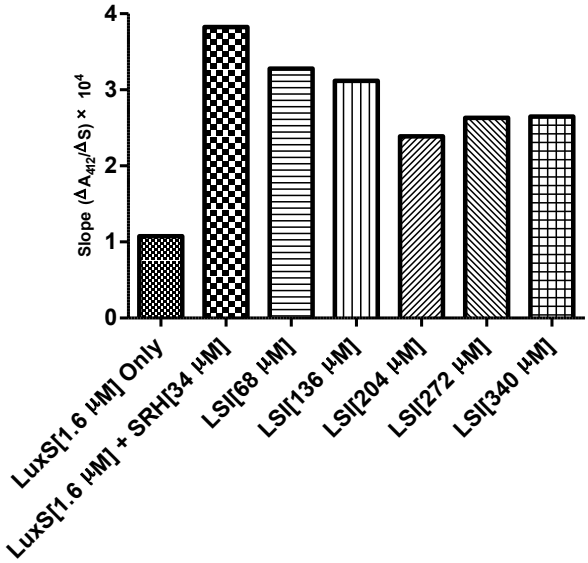
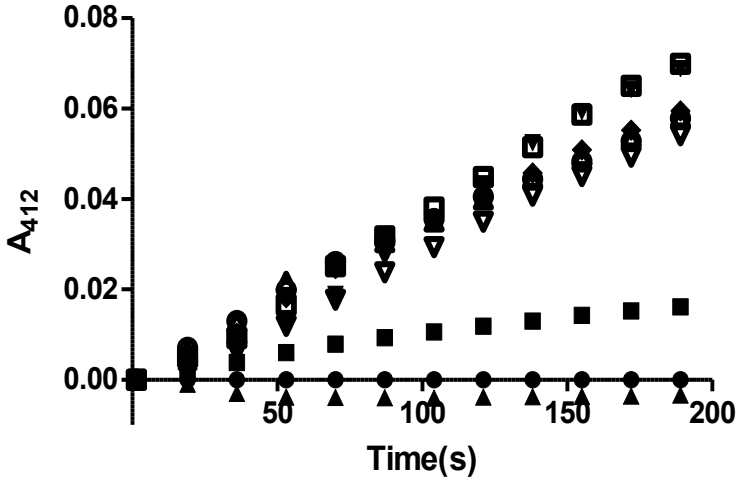
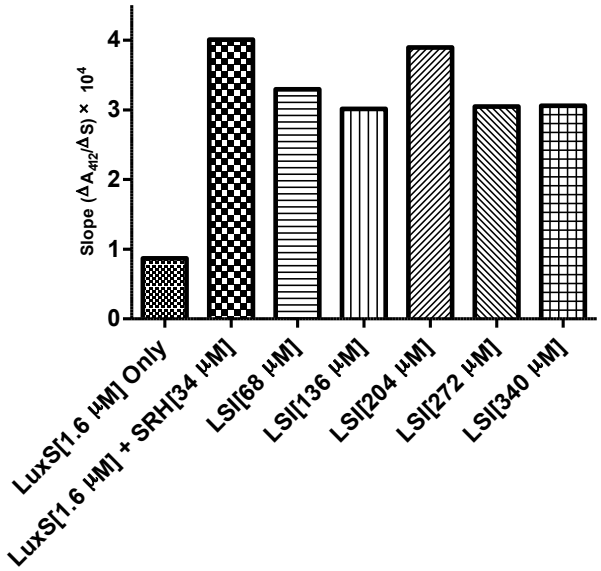


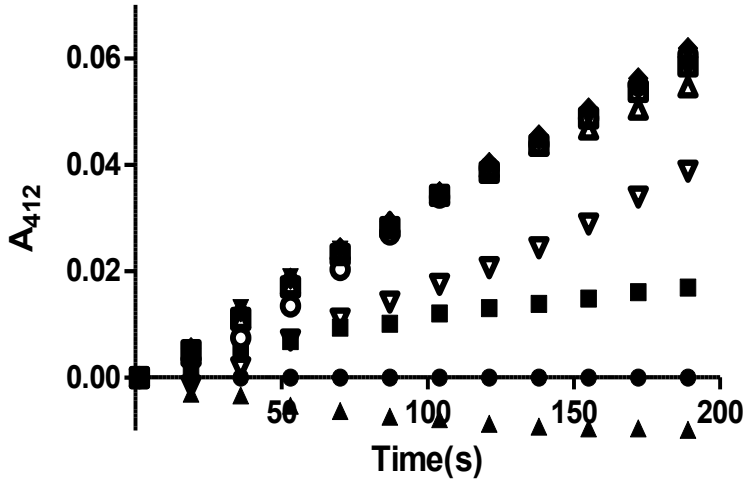
Figure 5 | L-Lysine. Graphs of all three LuxS Inhibition assay of L-Lysine.

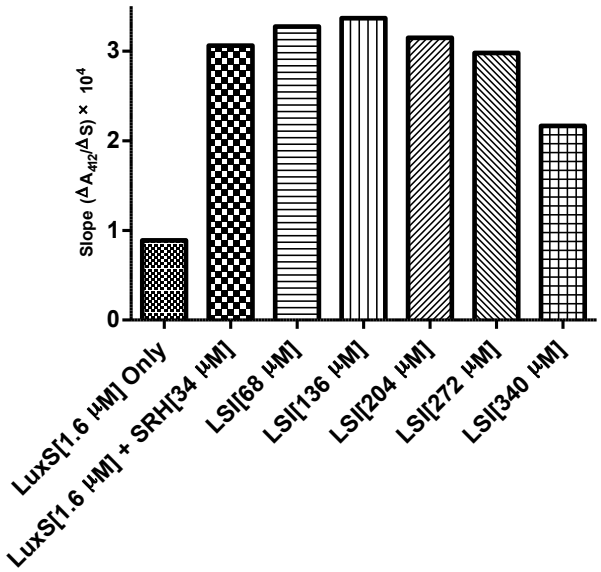
Succinic Acid Trial 1





Succinic Acid Trial 2





Succinic Acid Trial 3

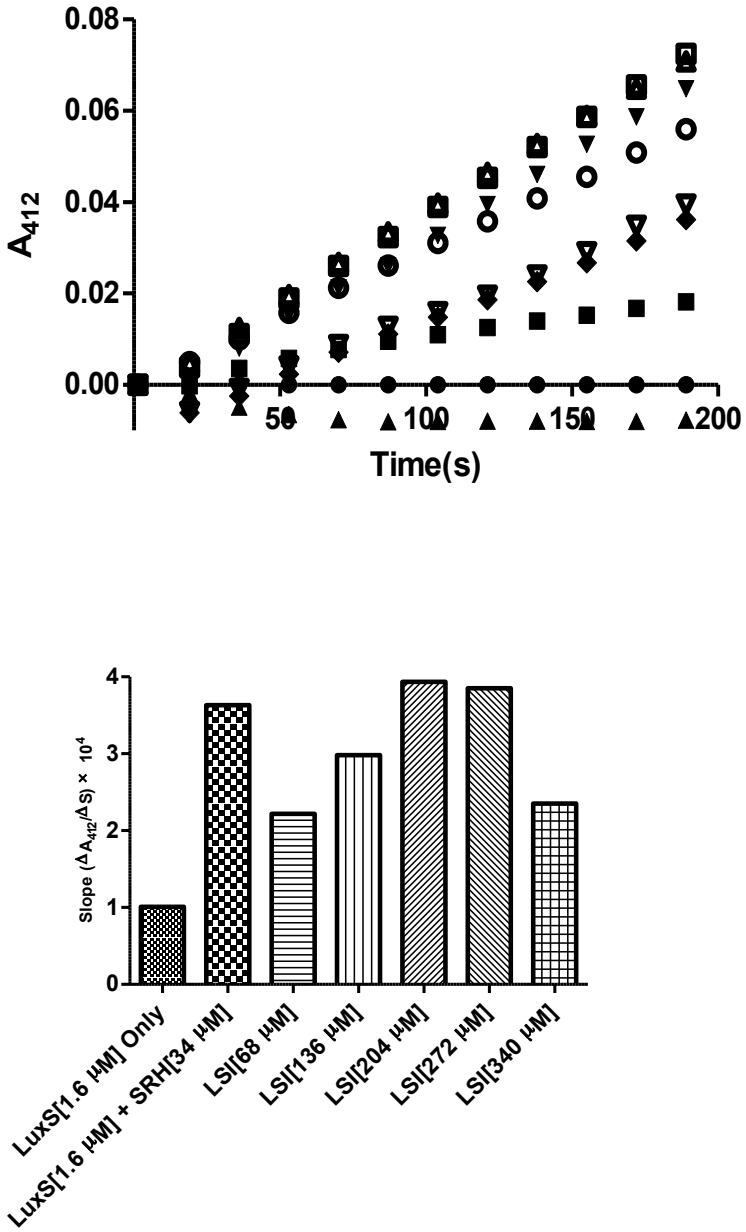
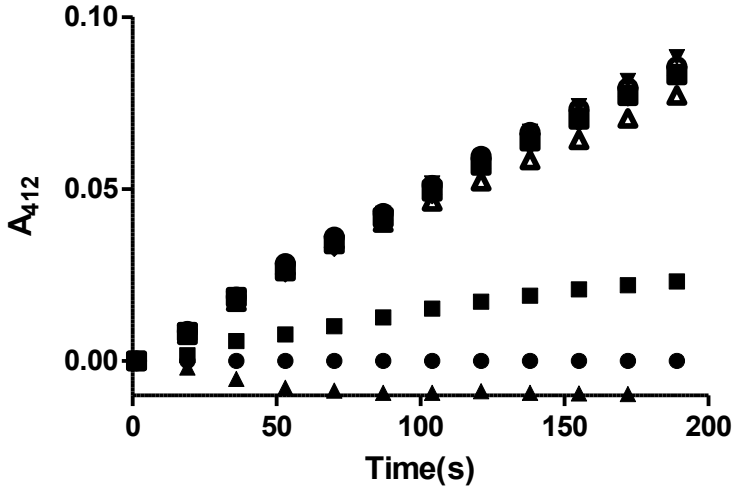
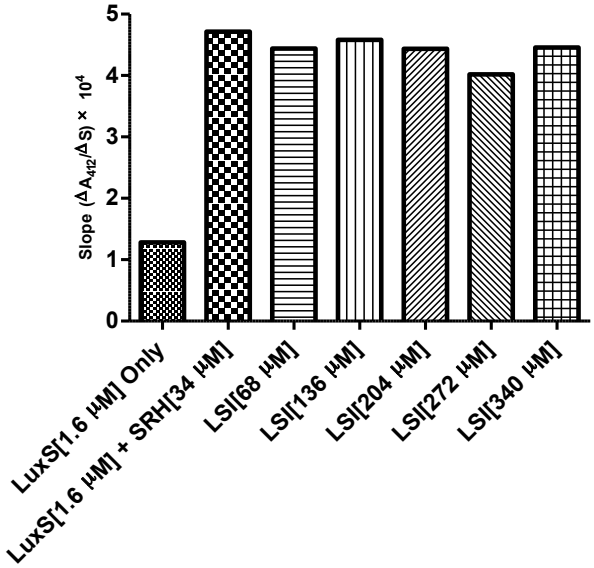


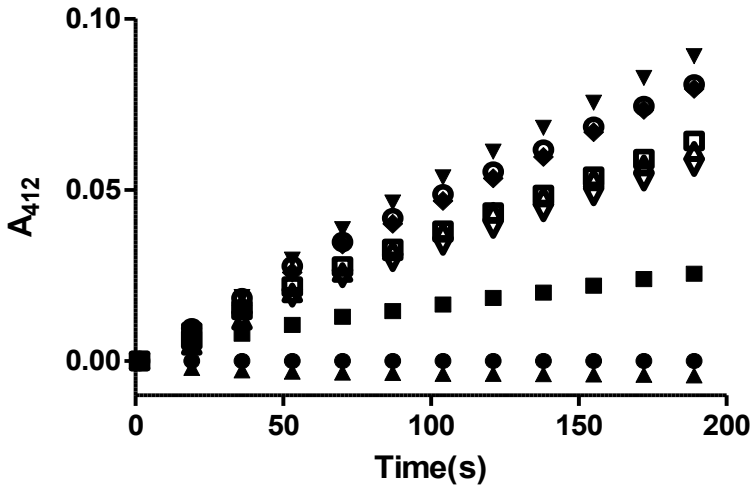
Figure 6 | Succinic Acid. Graphs of all three LuxS Inhibition assay of Succinic Acid.

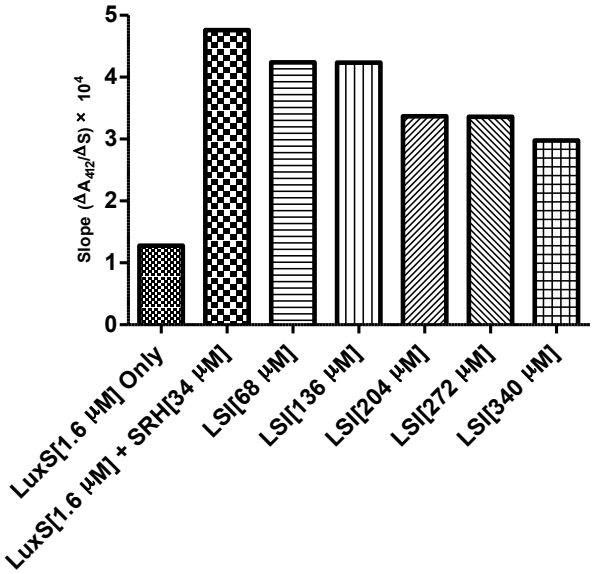
L-Glutamine Trial 1





L-Glutamine Trial 2





L-Glutamine Trial 3

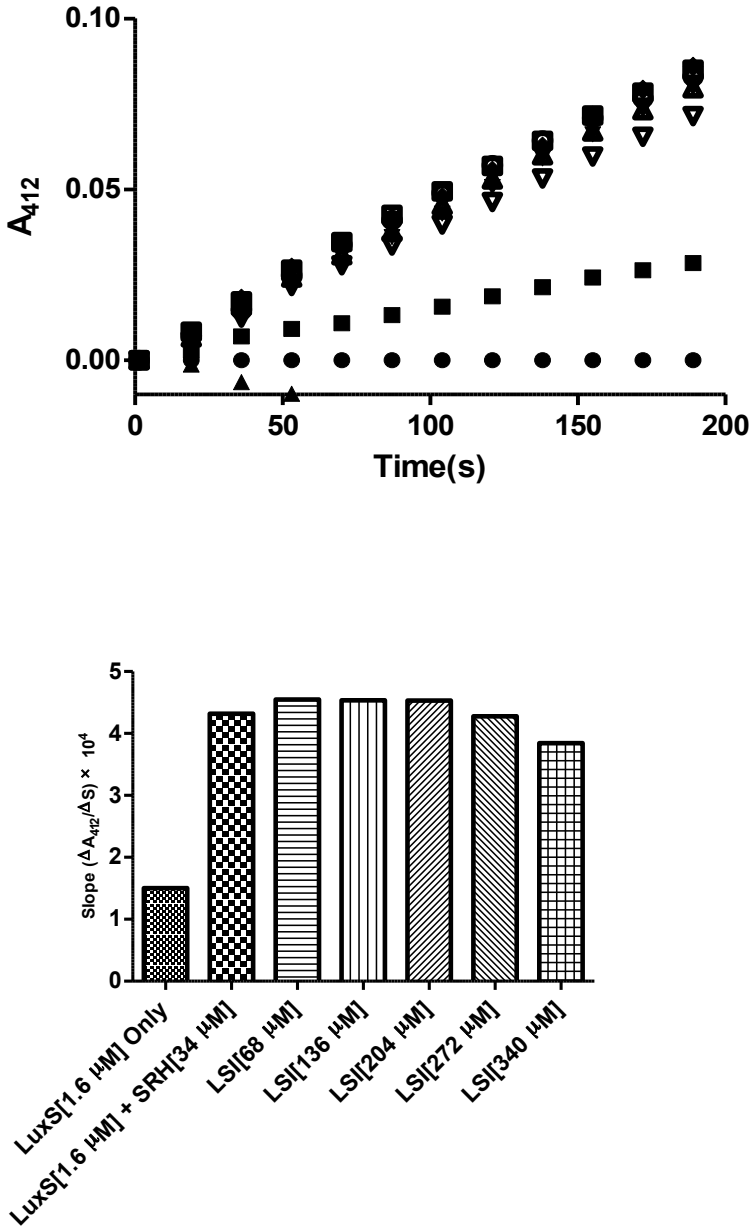
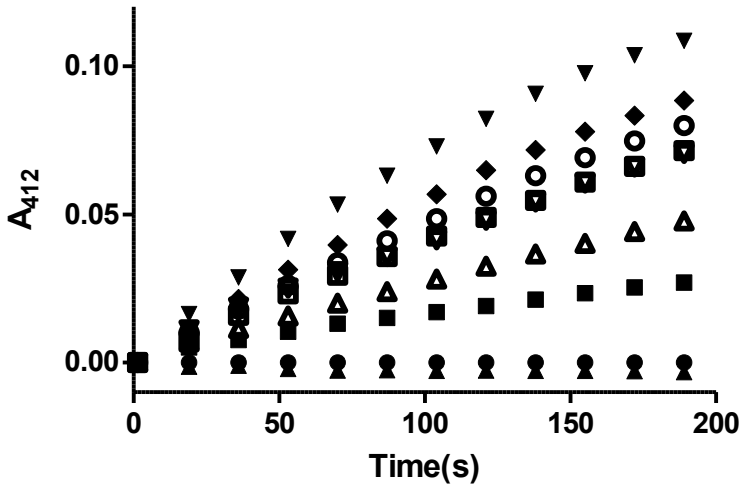
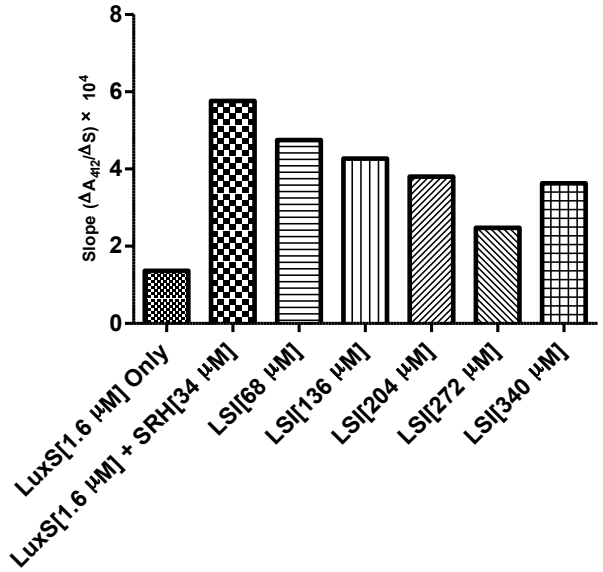


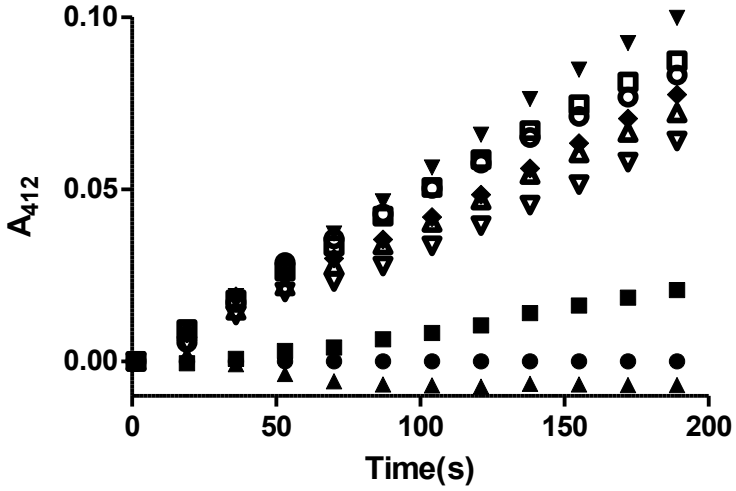
Figure 7 | L-Glutamine. Graphs of all three trials for the LuxS Inhibition assay of L-Glutamine.

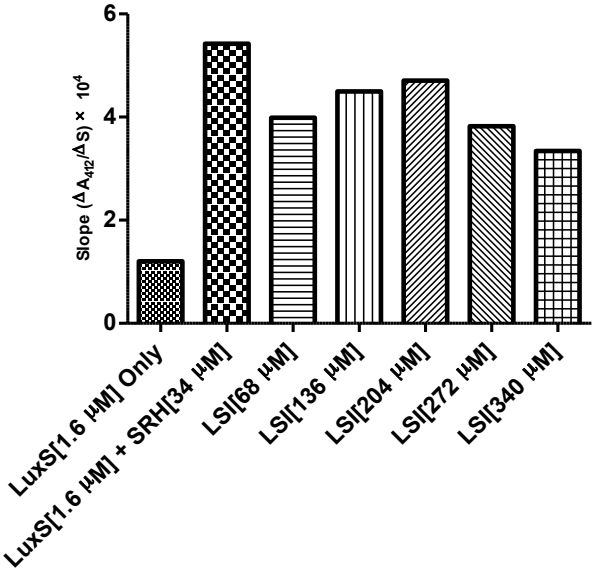
N^G, N^G- Dimethylarginine dihydrochloride Trial 1





N^G, N^G- Dimethylarginine dihydrochloride Trial 2





N^G, N^G- Dimethylarginine dihydrochloride Trial 3

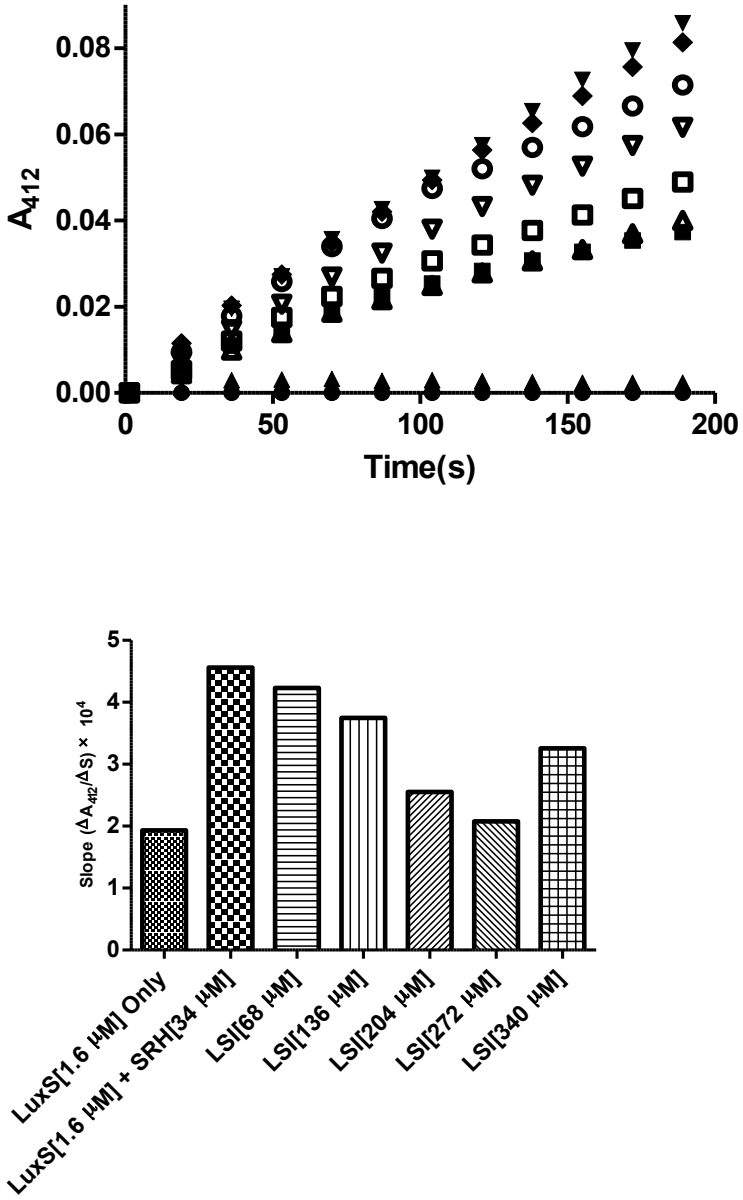
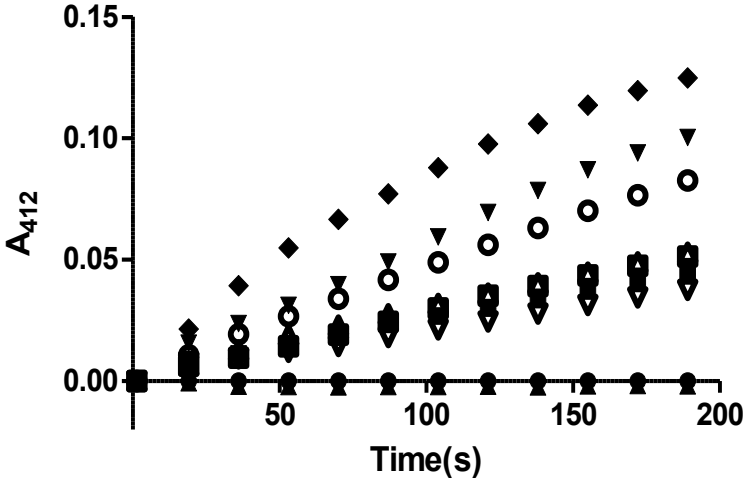
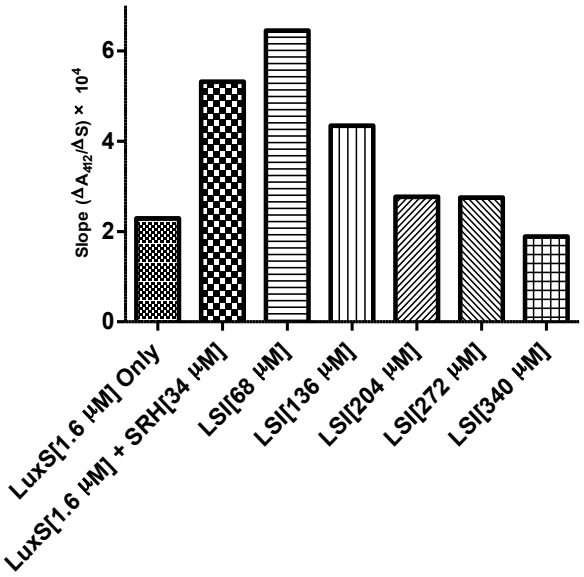


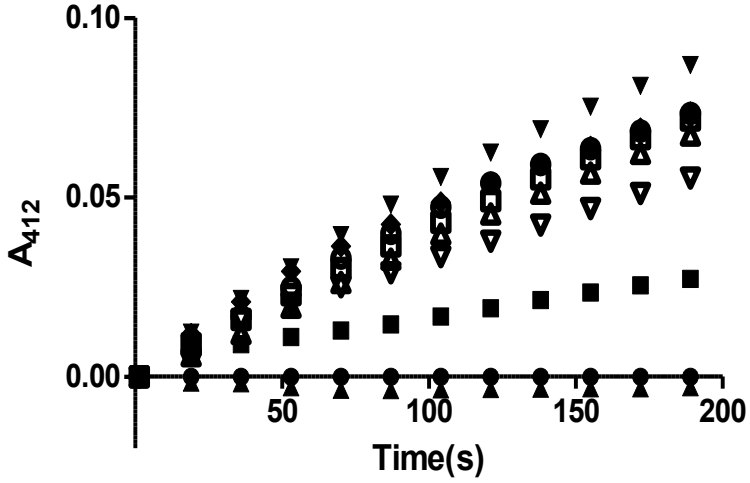
Figure 8 | N^G,N^G- Dimethylarginine dihydrochloride. Graphs of all three trials for the LuxS Inhibition assay of N^G,N^G- Dimethylarginine dihydrochloride.

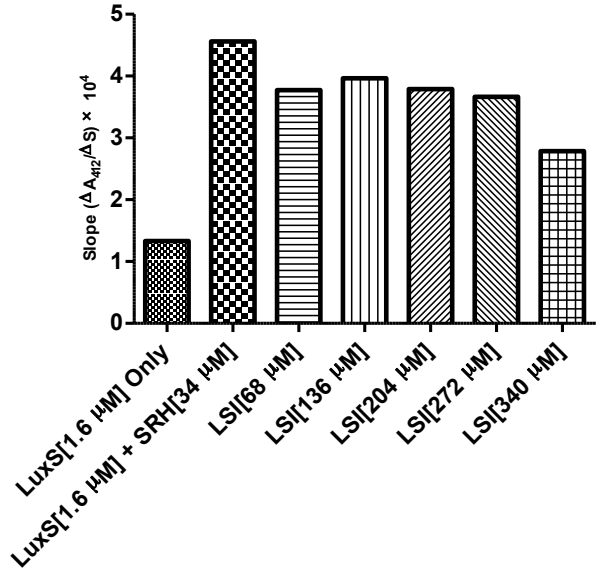
N^G-Methyl-L-arginine acetate salt (L-NMMA) Trial 1





N^G-Methyl-L-arginine acetate salt (L-NMMA) Trial 2





N^G-Methyl-L-arginine acetate salt (L-NMMA) Trial 3

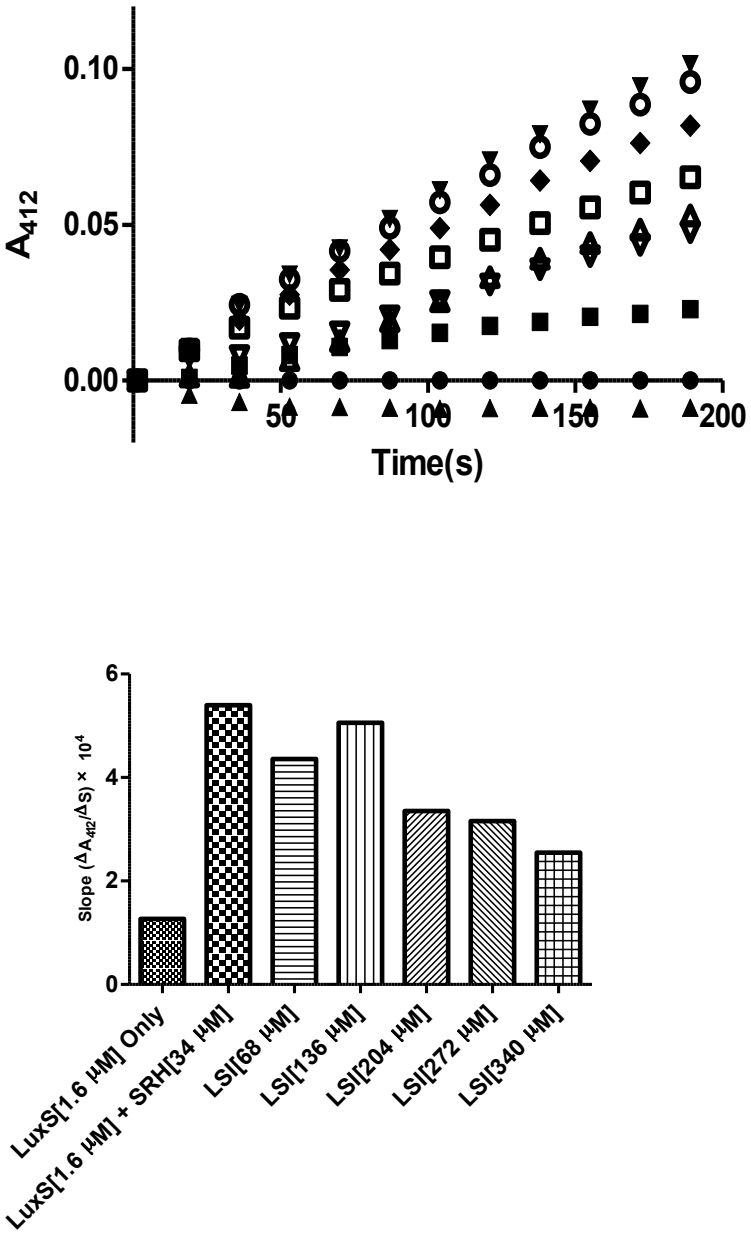
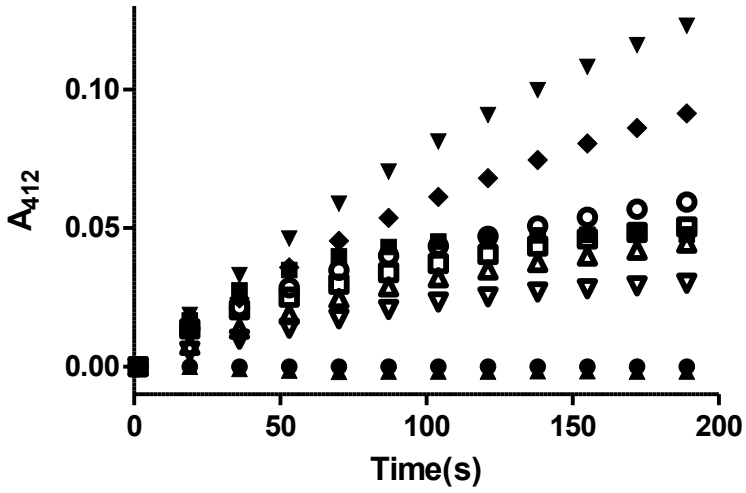
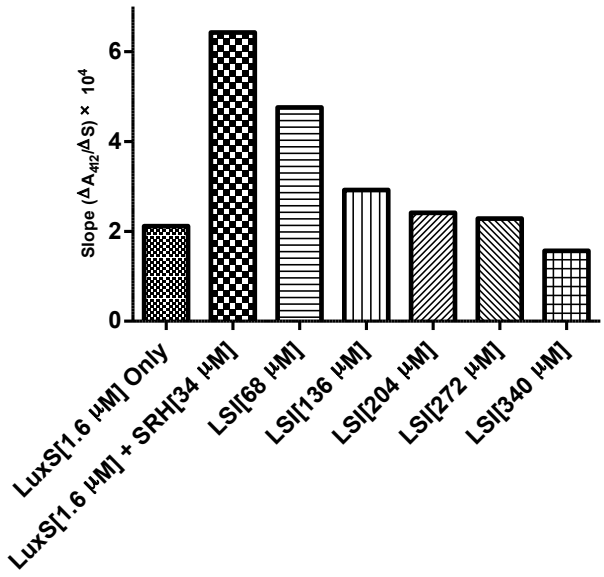


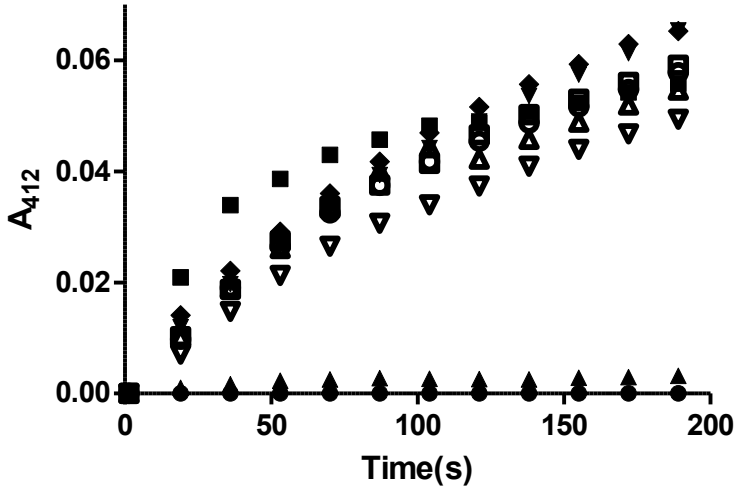
Figure 9 | N^G-Methyl-L-arginine acetate salt (L-NMMA). Graphs of all three trials for the LuxS Inhibition assay of N^G-Methyl-L-arginine acetate salt (L-NMMA).

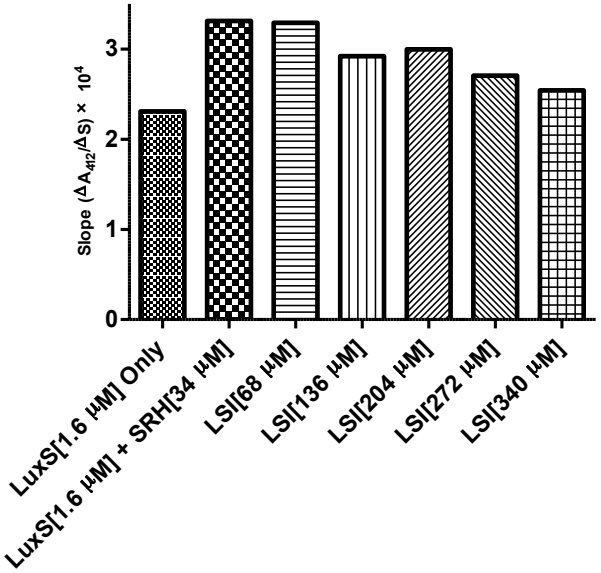
DL-Asparagine Trial 1





DL-Asparagine Trial 2





DL-Asparagine Trial 3

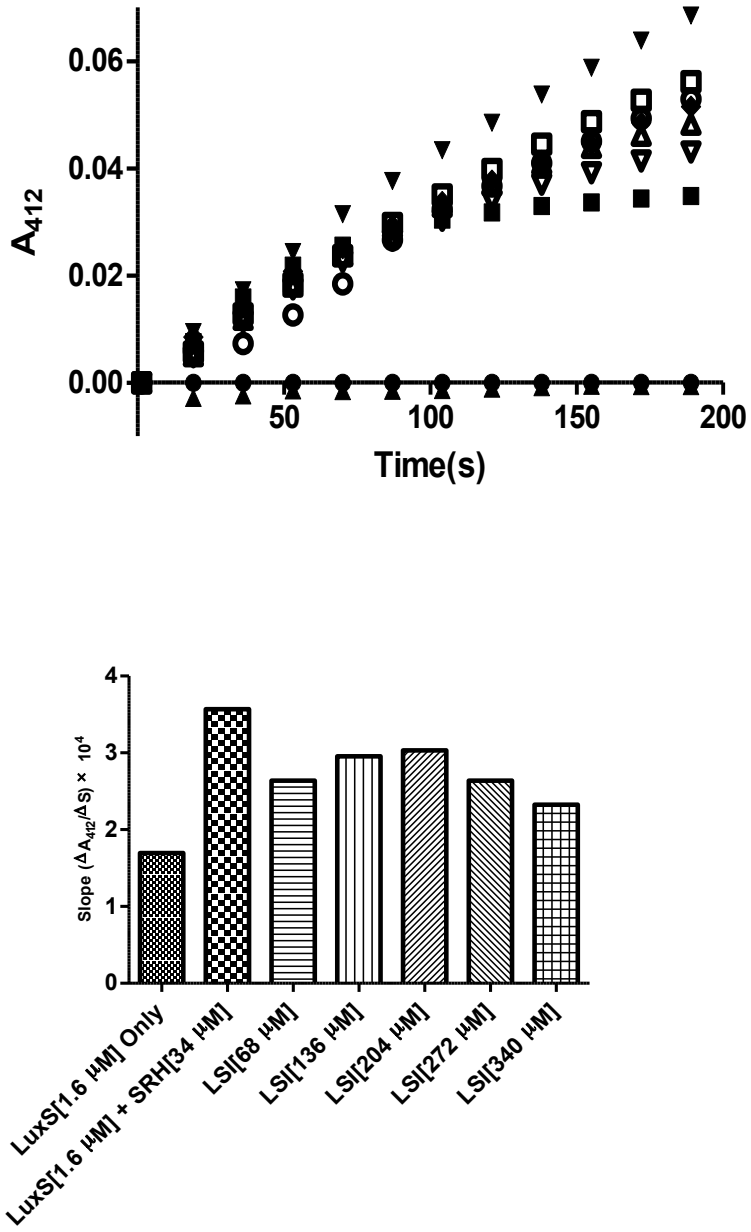
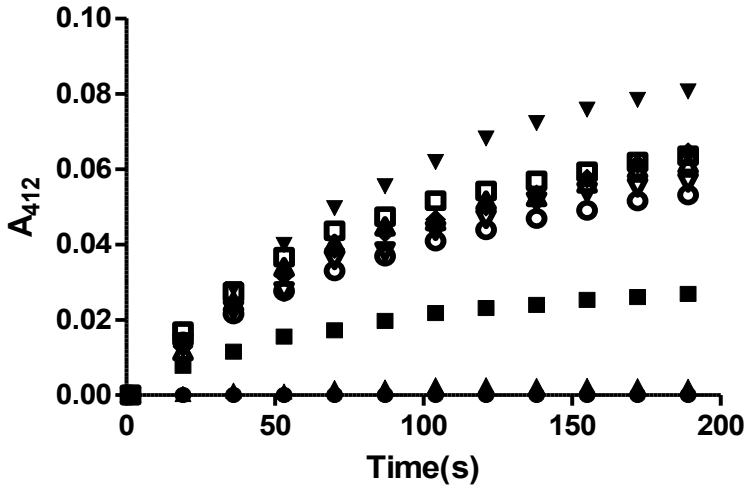
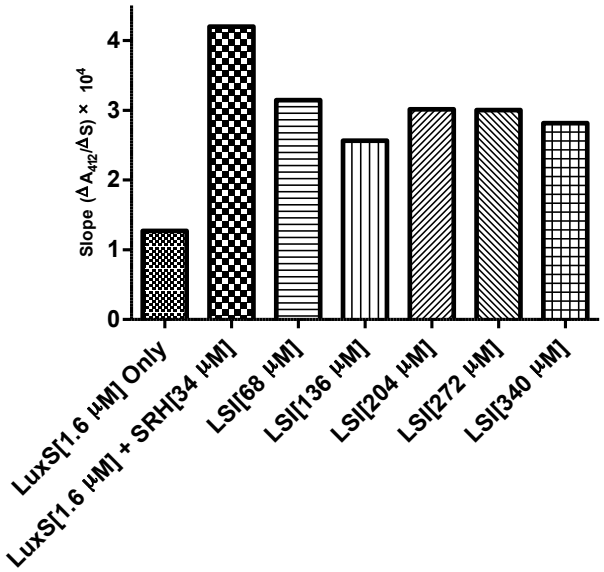


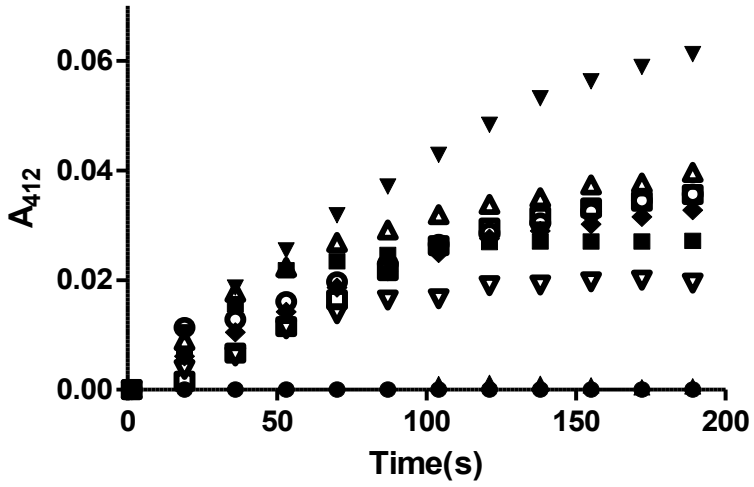
Figure 10 | DL-Asparagine. Graphs of all three trials for the LuxS Inhibition assay of DL-Asparagine.

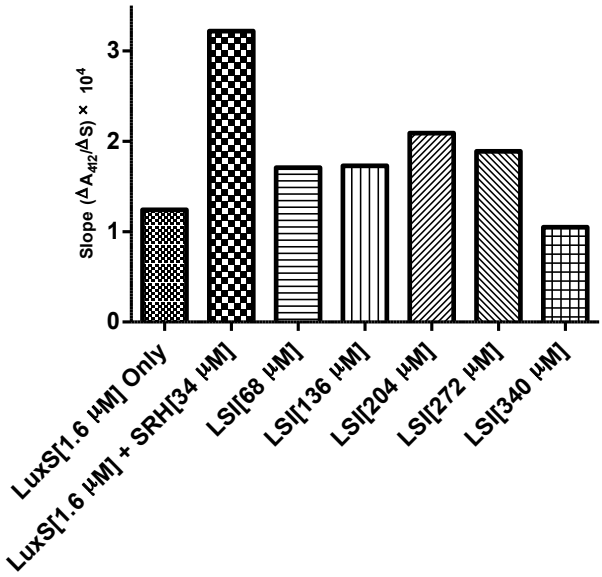
Sulfasalazine Trial 1





Sulfasalazine Trial 2





Sulfasalazine Trial 3

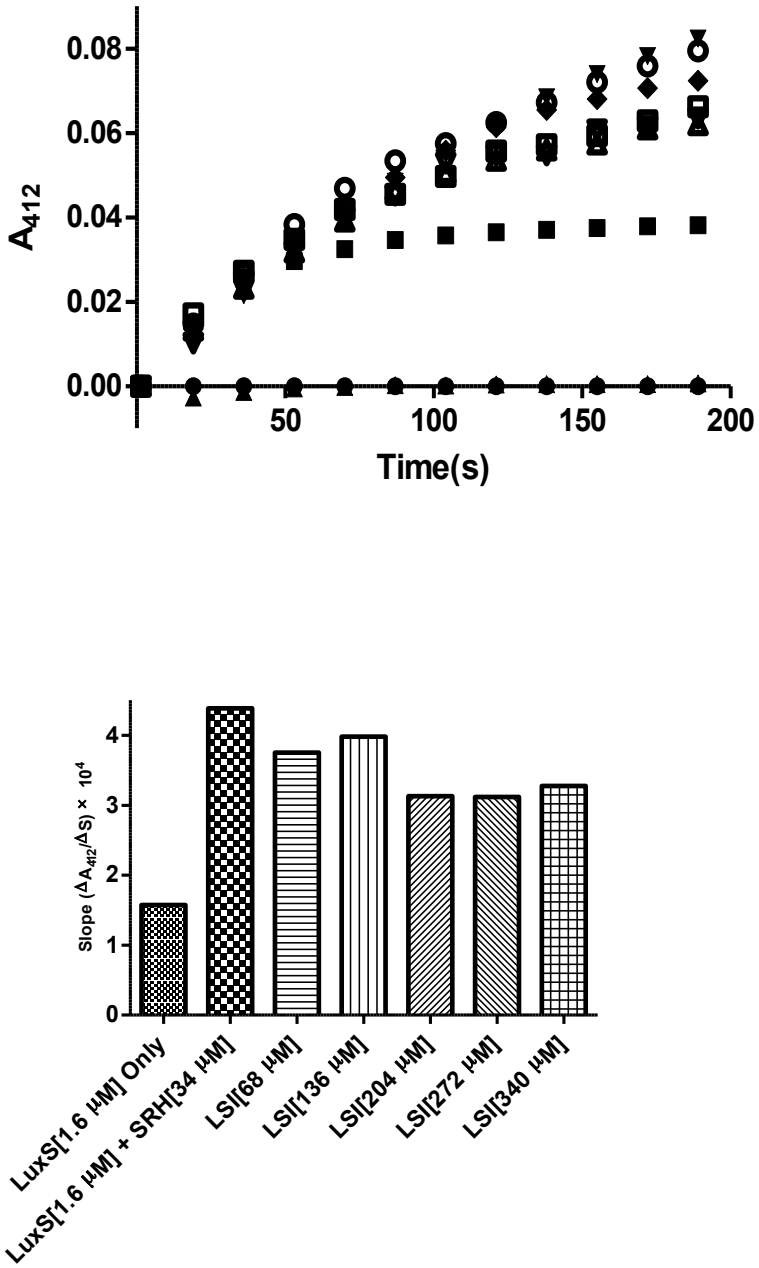
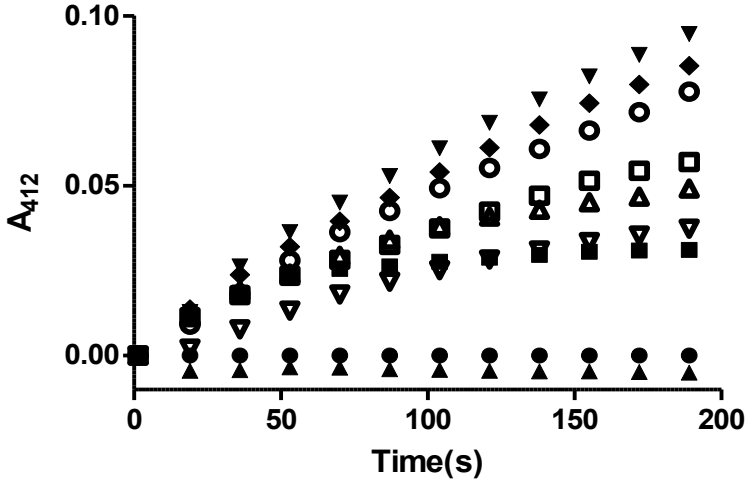
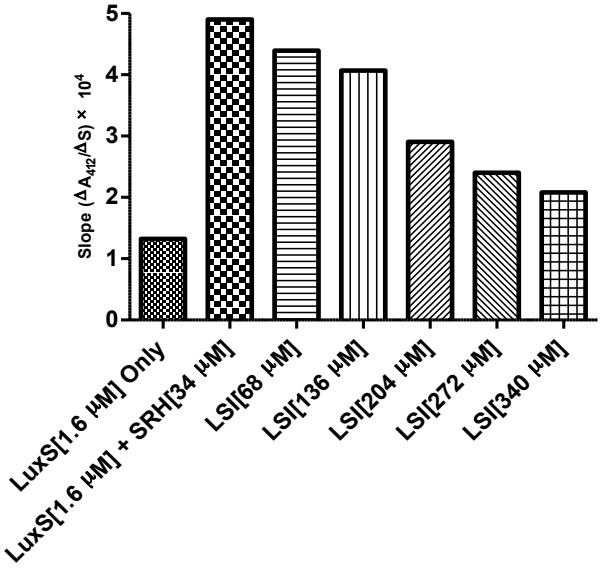


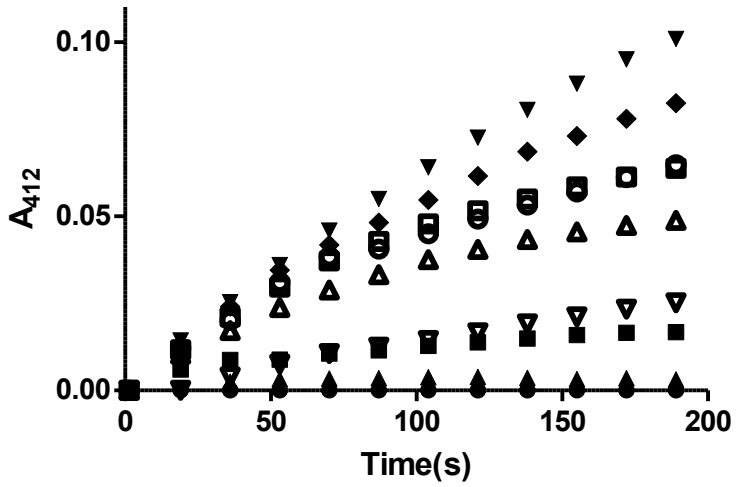
Figure 11 | Sulfasalazine. Graphs of all three trials for the LuxS Inhibition assay of Sulfasalazine.

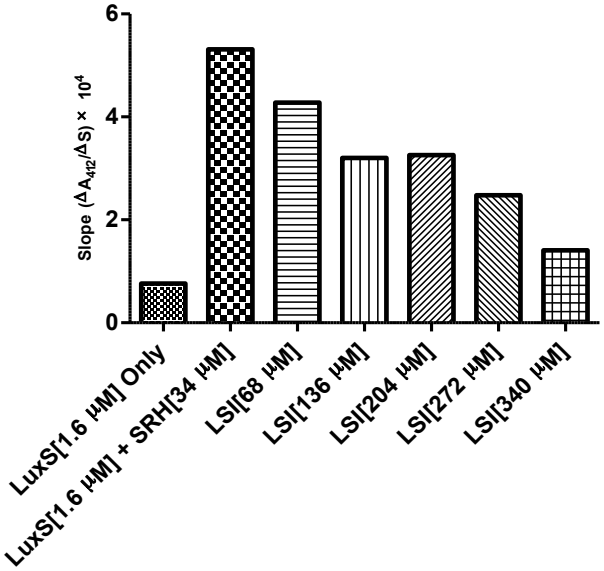
L-N^{G}-Nitroarginine methyl ester (L-NAME) Trial 1





L-N^{G}-Nitroarginine methyl ester (L-NAME) Trial 2





L-N^{G}-Nitroarginine methyl ester (L-NAME) Trial 3

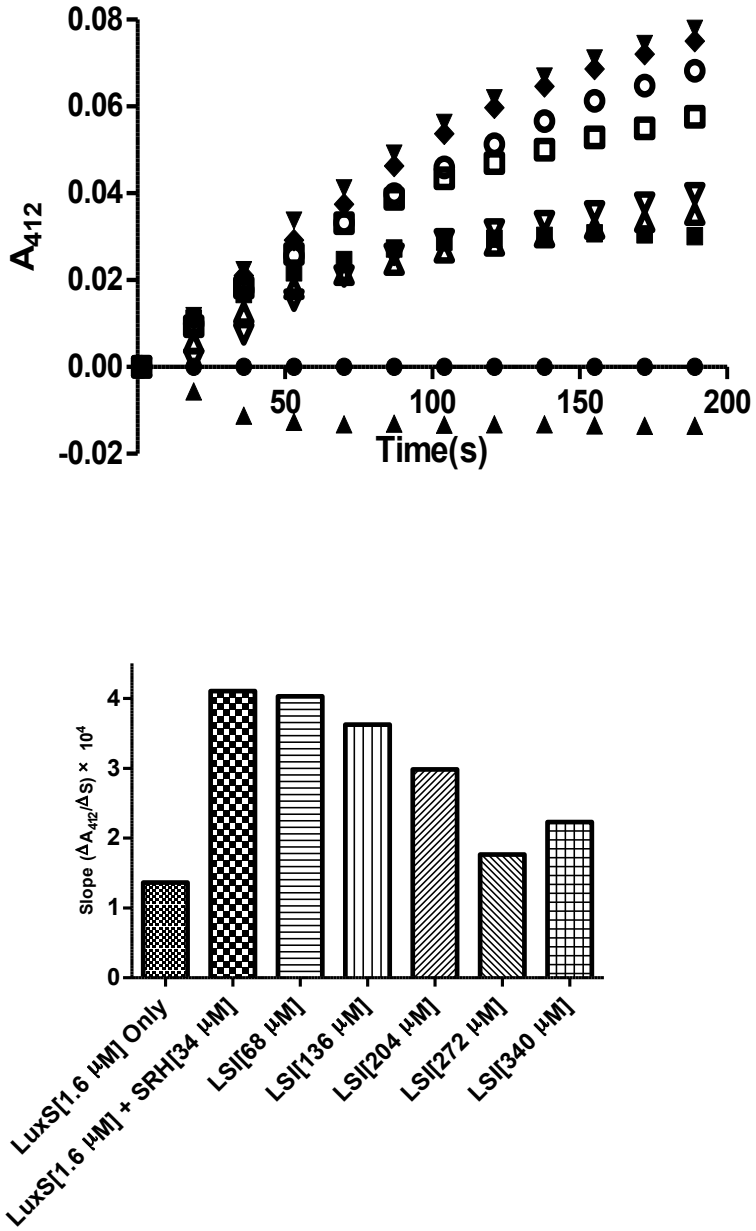


Figure 12 | L-N^{G}-Nitroarginine methyl ester (L-NAME). Graphs of all three trials for the LuxS Inhibition assay of L-NAME.

Appendix C: Sample Calculation Preparation

Sample Calculations for Inhibition Assays.

L-NAME Preparation:

Ellman's Reagent (15mM – 1mL stock)
o 0.005g DTNB
o 1000 µL 5× LuxS Buffer

5× LuxS Buffer- New Stock (Made 2/19/13)
o 19.5g of HEPES Sodium Salt (500mM)
o Adjust to pH 7.0 - HCl
o 6.5748g of NaCl (750mM)

SRH (680uM- 1000 µL stock)- Made from 20mM stock
o 200 µL 5× LuxS Buffer
o 34 µL SRH stock
o 766 µL ddH2O

Make **1M stock** of L-NAME
$0.01\text{g} \times \frac{\square ol269.7\ g}{} \times \frac{1\ L}{1\ mol} =$ **37.1 µL of 5× LuxS Buffer**

Make **680 µM stock of L-NAME**
(Stock used in assay)
(1 M)(x) = (0.00068 M)(2500 µL)
x = 1.7 µL of 1 M L-NAME stock
500 µL 5× LuxS Buffer
1998.3 µL dH_2O

Protein LuxS ELS1409 + glycerol (160µM- 120µL stock) – WT-LuxS_KW-2 (Assuming 1.75mM stock)
o 10.97 µL 5× LuxS Buffer
o 24 µL of ELS1409 (From 1.75 mM stock)
o 85.03 µL of ddH2O

Appendix D: Sample Activity Calculation for Ellman's and Inhibition Assays

Sample Calculation for Specific Acitivity in Ellman's assay or Inhibition assay.

$$2.20237 \times 10^{-4} \ \frac{AU}{s} \left(\frac{mol \cdot cm}{14000\ L}\right)\left(\frac{1}{cm}\right)\left(\frac{60\ s}{min}\right)\left(\frac{10^6\ \mu mol}{L \cdot min}\right)\left(\frac{1\ L}{1000\ mL}\right)\left(\frac{1\ mL}{0.0590872\ mg}\right)$$

= 0. 015974 μmol/mg/min